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KARYOTYPE ALTERATIONS IN HUMAN LUNG ADENOCARCINOMA CELLS AFTER LONG-TERM ACTION OF INTERFERON-ALPHA

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Aim: **To estimate the effect of long-term IFN treatment of human non-small-cell lung cancer cell line A-549 on their karyotype characteristics and on the clonal structure of cell population.** ***Methods:*** **Cytogenetic research was performed by standard methods using routine and differential staining. Cytogenetic characteristics were estimated per 1000 cells (ppm, (‰)).** ***Results:*** **Cytogenetic analysis of IFN-modified A-549 human lung cancer cells had demonstrated far-going changes in their population structure. It was shown that long term cultivation with IFN altered the chromosome modal class of A-549 cells, induced the domination of chromosomes with certain molecular markers: the number of metaphases with der (6) t (6; 1) chromosomal rearrangement increased significantly (from 6% to 80%, $p < 0.001$) and the cells with der (2) t (2; 1) markers almost disappeared. Thus, under the effect of IFN the cell clonal selection takes place. Decrease of the cell division rate and pseudometaphase occurrence, increase of the number of cells containing micronuclei are the typical characteristics of IFN-modified A-549 cell subline A-549IFN.** ***Conclusion:*** **Long-term IFN effect results in alterations of cytogenetic properties of A-549 human lung cancer cells.**
Key Words: **interferon-alpha, human lung cancer, cell culture, chromosomes.**

It is well-known that carcinogenesis and further tumor progression are based on cell genetic damage and genome instability. Structural chromosome alterations play a major role in tumor progression. Among the huge variety of chromosome anomalies in tumor cells, the specific or primary karyotype alterations are defined which are specific for certain tumor and leukemia types, and without any doubt are related to pathogenesis of these diseases. Structural malfunctions (translocations, deletions, inversions and amplifications) of oncogenes coding growth factors, cell receptors and other proteins, which control cell division and differentiation, are referred as primary chromosome alterations. Oncogene activation, tumor suppressor gene transfer, loss or damage, and also occurrence of structural DNA recombination due to translocation, which lead to forming of chimeric proteins, play the significant role in the neoplastic transformation and processes of tumor progression [1, 2].

Interferons (IFN) are typical representatives of so-called "biological reaction modifiers" which are incorporated into interaction between the tumor and the organism, providing non-specific anti-tumor defenses and enhancing specific organism-to-tumor response [3–5].

It is known that many IFN-induced genes belong to tumor suppressors [6–12]. On the other hand, integration of oncogenic virus DNA or cell transformation could be accompanied by damage of intracellular components of IFN system [10, 13–15]. IFNs can be referred to considerably small group of endogenous bioregulators, which can protect cells and their genome from various lesions. First of all, IFN is a classic anti-virus agent that prevents the replication of oncogenic virus and the integration of viral/proviral DNA into the cellular genome [16, 17]. In addition, previously it was demonstrated that IFN possesses apparent antimutagenic activity, and these studies derived a concept that "IFN is a cell genome guardian" [4, 18, 19]. However, those studies were not properly conducted, and the mechanisms of mentioned IFN effects and their role in carcinogenesis had not been studied yet.

Two main aspects of the problem of IFN participation in cell genetic consistence control can be derived. One of them, as have been mentioned previously, is related to the IFN's ability of direct participation in the control of cell genetic consistence. The other aspect is related to the genome stability asserted by IFN on a cell population level. That aspect is also insufficiently studied, while it is clearly demonstrated phenomenon in the clinical research of efficacy of IFN therapy in chronic myeloid leukemia (CML) patients.

It is shown in multiple studies that IFN therapy application results in complete hematologic as well as cytogenetic remission in about 70–80% of CML patients. An apparent alteration in cell population structure is detected in the blood of 30–40% of such patients [20]. This made possible to search for a new CML treatment tactics, switching from hematologic onset control to tumor clone suppression. That opportunity is confirmed by diminishing the levels of Ph+ cells and restoration of normal hemopoiesis if the IFN is used. While therapeutic efficacy of IFN in CML treatment is proven [20, 21], the precise mechanism of IFN-mediated tumor growth suppression remains unknown. So far, there was only one *in vitro* study, which demonstrated the unique ability of IFN to selectively inhibit the reproduction of Ph+ stem leukemic cells. IFN did not prevent the proliferation of normal bone marrow hematopoietic precursor cells. Probably, the mechanism of cytogenetic remission in CML patients during IFN therapy is in precise genetic selection of cell population [22]. Unfortunately, besides the wide use of IFN for the treatment of patients with solid tumors, these IFN-mediated effects have not been studied

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in the cells of solid tumors, in particular in the cells of non-small lung cancer.

At the time, if cells that occur spontaneously during neoplastic transformation loose sensitivity to various cytokines, this may lead to the accumulation of more aggressive tumor clones. For example, a deletion of chromosome I short arm is often found in solid tumors, and its presence is associated with tumor progression [23].

In current study we have performed a comparative analysis of A-549 cells and its subline A-549IFN that was modified by long-term action of IFN *in vitro,* to evaluate the ability of IFN to influence on karyotype characteristics of tumor cells and on the structure of cell population.

MATERIALS AND METHODS

Non-small cell lung cancer cell line (NSCLC) A-549 obtained from the Collection of Cell Lines of IEPOR NASU (Kiev, Ukraine) was cultured in complete RPMI 1640 medium supplemented with 4 mM L-glutamine, 10% fetal bovine serum, 40 μg/ml of gentamycine and 50 μg/ml of amphotericin B (Sigma, USA) in humidified atmosphere containing 5% CO_2, 37 °C. The concentration of IFN in A-549IFN cells media is 10 000 U/ml. The medium was replaced each two days, and cell passages were performed each four days with trypsin-versen solution. To study the long-term effect of IFN, the cells were cultivated for 1 year in the presence of recombinant IFN-alpha-2b (BioPharma, Ukraine) at increasing concentrations (from 100 to 10 000 U/ml) of INF. As a result, A-549 subline was obtained (A-549IFN) [7].

For a morphologic investigation, cytospin specimens were dried and Papenheim-stained. The levels of dividing cells, binuclear cells, cells containing micronuclei and apoptotic cells occurrence were calculated per 1000 cells and figured in per miles (‰). Chromosome specimens were prepared as it was described earlier [24].

Stained specimens were analyzed using Axiostar Plus microscope (Carl Zeiss, Germany) at x400–1000 magnification. Specimens of live and stained cells were photographed using Canon PowerShot G5 digital photo camera (Canon, Great Britain).

The statistical significance of the differences between mean values was assessed by the Student's *t*-test.

RESULTS AND DISCUSSION

An amount of chromosomes in the metaphase plates of A-549 cell culture varied from 15 to 63. Modal class in these cells comprised of 54–59 chromosomes; the majority of cells (28%) contained 56 chromosomes (Fig. 1). The characteristic feature of A-549 cell line is a giant submetacentric chromosome, which has been detected in more than 60% of observed metaphases (Fig. 2). Differential staining showed the marker chromosome as a result of translocation of a short arm chromosome 2 fragment onto a short arm of chromosome 1 (Fig. 3). This observation is consistent with the results of other studies of A-549 cell populations [25]. Nestor A.L. *et al.* [25] described among the marker chromosomes the most frequent translocations der (3) t (3, 20), der (6) t (6; 1), der (19) t (15; 19), + der (19) t (15; 19), del (2), der (11) t (8; 11). In current study we observed the presence of rearranged chromosomes previously described among the marker chromosomes of this line, as der (6) t (6; 1), der (11) t (8; 11), der (2) t (2, 1) in the metaphase plate of A-549 cells prior IFN treatment. Relatively low frequency (6% — 25%) of rebuilt chromosome der (6) t (6; 1) in the initial population cells A-549 should be noted.

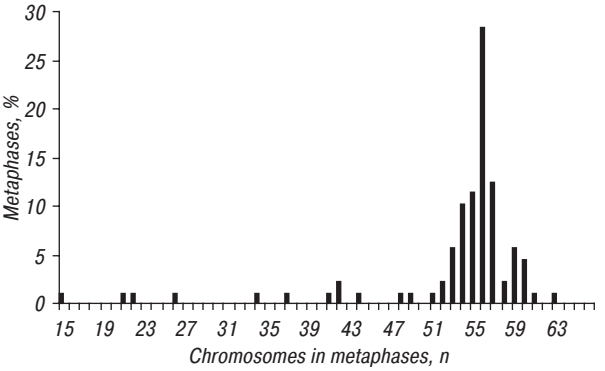

Fig. 1. Characteristics of modal class of chromosomes of A-549 cells

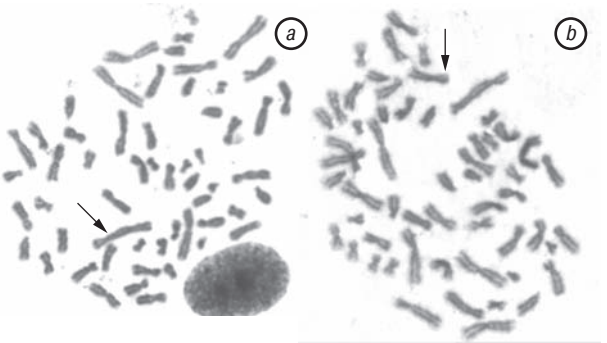

Fig. 2. Typical metaphase plates of A-549 cells with giant submetacentric chromosomes (arrows)

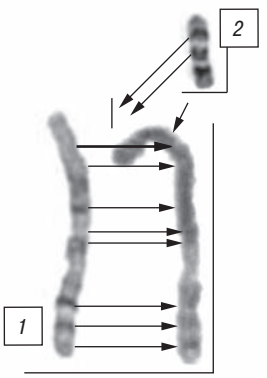

Fig. 3. Differential staining of marker chromosome with translocation t (1; 2) in the cell clones dominated in the parental cell line A-549

Studies conducted using the method of fluorescence *in situ* hybridisation of DNA probes to different chromosomes showed a high initial heterogeneity of A-549 cell population on the marker chromosomes [26]. It is estimated that about 25% of chromosomes of this line are involved in various chromosomal rearrangements. It was shown previously that such rearrangements results in marked increase of chromosome 1 copies compared to other autosomes (5.8 ± 1.4 copies of chromosome 1 in the karyotype)

[26]. The high degree of heterogeneity of rearranged marker chromosomes in A-549 cell population makes it difficult to allocate some marker chromosomes or chromosome combinations among them [25, 26]. At the same time, translocation of the long arm of chromosome 1, which is most easily identified by differential staining, allows to track the impact of various external factors on the structure of the cell population.

The experiments performed earlier described the data of incorporation of chromosome 1 in intra- and interchromosomal recombination in the tumor cells of various origins. Thus, for example, patients with germinogenic tumors often have normal karyotype, but an aberration in chromosome 1 occurs frequently, for example, the short arm of chromosome 1 can be doubled or lost [27]. The most frequent chromosomal aberration in neuroblastoma patients is a deletion of chromosome 1 short arm — del (1p) [23].

Translocation 1; 2 (q25; p23) is detected in some anaplastic giant cell lymphoma patients [28]. Authors suggest that the *Tmp3* gene on the chromosome 1 (q25) and anaplastic lymphoma kinase (*ALK*) gene on 2p23 arm are incorporated in this translocation [29], and the translocation is accompanied by the expression 104 kDa chimera protein TMP3-ALK [28].

The A-549IFN cell line was derived from original A-549 cell line by long-term cultivation in the presence of IFN. Previously we have shown that prolonged treatment of A-549 cells with IFN leads to significant changes in their phenotypic properties, many of which persist for a long time (up to two months) even in the absence of IFN [30]. Therefore, we proposed that this could not be the result of constant induction of genes expression by IFN: new features of A-549IFN cells could be explaned by stable genetic and epigenetic changes due to IFN-induced selection of cell population.

Cytogenetic analysis of IFN-modified cells (A-549IFN), held in the dynamics at different stages of new subline formation, showed profound changes in the clonal composition of these cells. It was shown that in new cell population modal class of chromosomes varies: an amount of chromosomes in the metaphase plates of A-549IFN cell culture varied mostly from 48 to 66, modal class in these cells included 58–62 chromosomes (Fig. 4). A majority of these cells contained 62 chromosomes. In the cells dominating in the population changes occured with a specific marker chromosomes: the number of metaphases with chromosomal rearrangement der (6) t (6, 1) greatly increased (from 6% to 80%, $p < 0.001$), and cells with marker der (2) t (2, 1) almost disappeared from population (see Fig. 3; Fig. 5). Thus, these data demonstrate the process of clonal selection in A-549 cells under the long-term action of IFN, like that of described earlier for CML cells [22]. A typical feature of the A-549IFN subline is a significant decrease in frequency of cell division and pseudometaphases formation ($p < 0.001$), and small increase of number of cells with micronuclei (Table). The higher sensitivity of these cells to apoptosis demonstrated in previous studies [30] could explain current observations. It was found that cultivation of IFN-modified A-549IFN cells in the absence of IFN for 45 days was accompanied by further cytogenetic and phenotypic unification of the population — the dominance of clones with markers der (6) t (6, 1), modal number of 54–59 chromosomes in 80% of cells. Observed characteristics of chromosome modal class indicate the decrease of aneuploidy in the new cell population of A-549IFN line. This is in line with previous data on the decrease of indices of malignant phenotype of these cells compared with parental A-549 line [30].

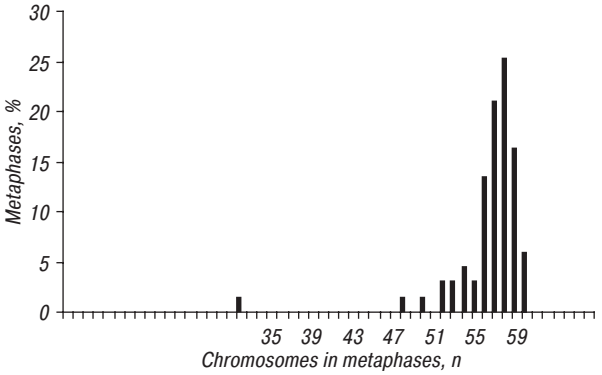

Fig. 4. Characteristic of modal class chromosomes in A-549IFN cells

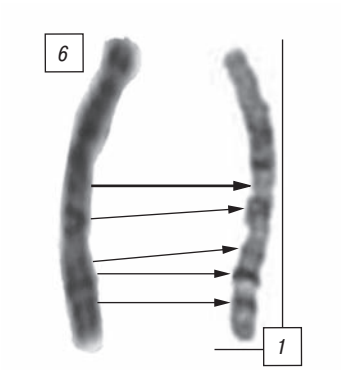

Fig. 5. Differential staining of marker chromosome with translocation t (6, 1) in the cell clones dominated in A-549IFN subline

Table. The frequency of occurrence of cell division, pseudometaphases and cells with micronuclei and apoptotic cells in A-549 and A-549IFN cell line

Cell line	Number of studied cells	Frequency of occurrence (‰)			
		Pseudometaphases	Mitosis	Micronuclei	Apoptosis
A-549	8000	5.6 ± 1.0	12.2 ± 1.9	2.6 ± 0.8	4.5 ± 1.5
A-549IFN	7000	1.4 ± 0.9*	3.6 ± 0.8*	5.7 ± 1.4	1.9 ± 0.5

*$p < 0.001$.

Thus, obtained results directly demonstrate that in the presence of IFN clonal selection take place in the original cell population and gives rise to a new subline, which differs from the original line by a number of biological and phenotypic parameters describe earlier [30]. Karyological changes that we identified in A-549IFN cells, as well as continuous changes in the expression level of several protein markers indicate the presence of both the adaptive and selective mechanism in the reaction of cells in response to IFN [31, 32]. The cell system, which was developed, could be perspective to reveal the role of adaptive and selective mechanisms in the emergence of a new, less malignant cell clones under long-term action of IFN.

REFERENCES

1. **Knudson AG.** Mutation and cancer: a personal odyssey. Adv Cancer Res 1995; **67**: 1–23.

2. **Weinberg RA.** Oncogenes and tumor suppressor genes. CA Cancer J Clin 1994; **44**: 160–70.

3. **Brierley MM, Fish EN.** IFN-alpha/beta receptor interactions to biologic outcomes: understanding the circuitry. J Interferon Cytokine Res 2002; **22**: 835–45.

4. **Vilcek J.** Fifty years of interferon research: aiming at a moving target. Immunity 2006; **25**: 343–8.

5. **Vorontsova AL, Kudryavets YuI, Zhylchuk VE.** Interferons and their application in clinical oncology. Women Health 2003; **4**: 8–14 (In Russian).

6. **Meurs EF, Galabru J, Barber GN, *et al.*** Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. Proc Natl Acad Sci USA 1993; **90**: 232–6.

7. **Stadler M, Chelbi-Alix MK, Koken MHM, *et al.*** Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. Oncogene 1995; **11**: 2565–73.

8. **García MA, Collado M, Muñoz-Fontela C, *et al.*** Antiviral action of the tumor suppressor ARF. EMBO J 2006; **25**: 4284–92.

9. **Garcia MA, Gil J, Ventoso I, *et al.*** Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action. Microbiol Molec Biol Rev 2006; **70**: 1032–60.

10. **Calo V, Migliavacca M, Bazan V, *et al.*** STAT proteins: from normal control of cellular events to tumorigenesis. J Cell Physiol 2003; **197**: 157–68.

11. **Kroger A, Dallugge A, Kirchhoff S, *et al.*** IRF-1 reverts the transformed phenotype of oncogenically transformed cells *in vitro* and *in vivo*. Oncogene 2003; **22**: 1045–56.

12. **Choo A, Palladinetti P, Passioura T, *et al.*** The role of IRF1 and IRF2 transcription factors in leukaemogenesis. Curr Gene Ther 2006; **6**: 543–50.

13. **Wood A, Angus B, Kestevan P, *et al.*** Alpha interferon gene deletions in post-transplant lymphoma. Br J Haematol 1997; **98**: 1002–3.

14. **Rincon-Orozco B, Halec G, Rosenberger S, *et al.*** Epigenetic silencing of interferon-kappa in human papillomavirus type 16-positive cells. Cancer Res 2009; **69**: 8718–25.

15. **Zekri AR, Moharram RA, Mohamed WS, *et al.*** Disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma is associated with repression of interferon regulatory factor-1. Eur J Gastroenterol Hepatol 2010; **22**: 450–6.

16. **Huleihel M, Aboud M.** Inhibition of retrovirus DNA supercoiling in interferon-treated cells. J Virol 1983; **48**: 120–6.

17. **Rady PL, Schnadig VJ, Weiss RL, *et al.*** Malignant transformation of recurrent respiratory papillomatosis associated with integrated human papillomavirus type 11 DNA and mutation of p53. Laryngoscope 1998; **108**: 735–40.

18. **Gubitskaia EG, Sinel'shchikova TA, Akhmatullina NB, *et al.*** Optimization of protective properties of interferons in human cells exposed to mutagens estimated by the DNA repair activity. Genetika 1990; **26**: 1519–22 (In Russian).

19. **Fomenko L, Sirota N, Ravin V, *et al.*** Interferon alpha reduces the level of radiation-induced micronuclei in mouse bone marrow cells. Arch Immunol Ther Exp 1997; **45**: 475–8.

20. **Jabbour E, Fava C, Kantarjian H.** Advances in the biology and therapy of patients with chronic myeloid leukaemia. Best Pract Res Clin Haematol 2009; **22**: 395–407.

21. **Kawakubo K, Ohyashiki K, Ohyashiki JH, *et al.*** A possible correlation between interferon-stimulated gene expression and cytogenetic responses in chronic myelogenous leukemia patients treated with alpha-interferon. Jpn J Clin Oncol 1996; **26**: 59–64.

22. **Cornelissen JJ, Ploemacher RE, Wognum BW, *et al.*** An *in vitro* model for cytogenetic conversion in CML. Interferon-alpha preferentially inhibits the outgrowth of malignant stem cells preserved in long-term culture. J Clin Invest 1998; **102**: 976–83.

23. **Polischuk LZ.** The chromosome alterations and human malignant tumors. Cytology and Genetics 1988; **4**: 65–72 (In Russian).

24. **Kovaleva OA, Glazko TT, Kochubey TP, *et al.*** Spontaneous premature condensation of chromosomes in normal and transformed mammal cells. Exp Oncol 2007; **29**: 18–22.

25. **Nestor AL, Hollopeter SL, Matsui SI, *et al.*** A model for genetic complementation controlling the chromosomal abnormalities and loss of heterozygosity formation in cancer. Cytogenet Genome Res 2007; **116**: 235–47.

26. **Isaka T, Nestor AL, Takada T, *et al.*** Chromosomal variations within aneuploid cancer lines. J Histochem Cytochem 2003; **51**: 1343–53.

27. **Mamaev NN, Mamaeva SE, Ushakova EA, *et al.*** The results of the activity studies of nucleolar organizers in bone marrow cells of multiple myeloma patients. Cytology and Genetics 1988; **2**: 91–7 (In Russian).

28. **Gluzman DF, Nadgornaya VA, Sklyarenko SV, *et al.*** Cytological variants of non-Hodgkin lymphomas in children. Oncology 2005; **7**: 320–6 (In Russian).

29. **Lamant L, Dastugue N, Pulford K, *et al.*** A new fusion gene TPM3-ALK in anaplastic large cell lymphoma created by a (1;2)(q25;p23) translocation. Blood 1999; **93**: 3088–95.

30. **Kudryavets YuI, Bezdenezhnykh NO, Lukyanova NYu, *et al.*** Modifying influence of prolonged action of interferon on phenotypic characteristics of human lung cancer cells *in vitro*. Exp Oncol 2008; **30**: 283–8.

31. **Scheel C, Onder T, Karnoub A, *et al.*** Adaptation versus selection: the origins of metastatic behavior. Cancer Res 2007; **67**: 11476–9.

32. **Mahale AM, Khan ZAT, Igarashi M, *et al.*** Clonal selection in malignant transformation of human fibroblasts transduced with defined cellular oncogenes. Cancer Res 2008; **68**: 1417–26.