[Frontiers in Bioscience 11, 1844-1853, May 1, 2006]

Molecular cytogenetic characterization of a novel cell line established from a superficial spreading melanoma

Andrea Treszl [1], Andrea Ladanyi [2], Zsuzsa Rakosy [1], Zsuzsa Buczko [3], Roza Adany [1] and Margit Balazs [1]

[1] Department of Preventive Medicine, School of Public Health, Medical and Health Science Centre, University of Debrecen, Debrecen, Hungary, [2] Department of Tumor Progression, National Institute of Oncology, Budapest, Hungary, [3] Department of Obstetrics and Gynecology, Medical and Health Science Centre, University of Debrecen, Debrecen, Hungary

TABLE OF CONTENTS

1. ABSTRACT

We report the complex cytogenetic analysis of a novel melanoma cell line (M35/01) established from a vertical growth phase of a superficial spreading melanoma. Similarly to its parental tumor, this cell line metastasizes to the liver. Using combined molecular cytogenetic techniques, we could identify a reservoir of chromosomal alterations in M35/01. In addition, we had sufficient amount of DNA from both the original primary tumor and the cell line which allowed for the comparison of their genetic patterns by chromosomal CGH. Several common alterations were found indicating the same clonal origin. These alterations included gains of 6p, 7q, 15q and deletions of 9, 10, 16q and 17p. Chromosomal losses present only in the cell line were detected on chromosome 4, 16p, 18 and gains on 20p12-qter. Array CGH analysis of the M35/01 cell line provided similar results with a much higher resolution, representing relatively high level gains on 7q31.2-q31.31, 15q25, 20q, and losses on 4q28, 9p21-p24, 9q21-q22, 10q25, 16q13-q23, 17p12-13 and 18q12-23. Using SKY-FISH, several structural alterations could be detected which were not recognized by conventional cytogenetics. Except for chromosome 18, none of the centromeres showed normal distribution by FISH. Our analysis shows that a high number of chromosomal alterations, which are known to be nonrandomly associated with melanoma progression, can be found by the combined use of different molecular genetic techniques. This new melanoma cell line would be an excellent model for investigating the mechanism of organ specific-metastatic events of malignant melanoma.

2. INTRODUCTION

The development of malignant melanoma is a very complex and still not fully understood process. In addition to periodic, high-dose solar UV exposure and the related immune response, other factors including genetic predisposition, skin pigmentation, mutations in DNA repair genes and alteration of cell cycle regulators also play an important role during the evolution of this malignancy (1). High risk surgically resected melanoma is associated with 5-year survival less than 50%, and as soon as the first distant metastasis appears, the disease becomes one of the most aggressive and chemoresistant tumors; 90-95% of patients suffering from melanoma metastasis do not survive for more than 3 years (2, 3).

The generation of melanoma cell lines established from primary tumors is an essential tool for clarifying the molecular background of the biological behavior of malignant melanoma. Cell lines that metastasize to known organs can help to investigate the molecular mechanisms of the metastatic process and might be of clinical significance in terms of developing better therapeutic strategies, thereby improving patients' survival. However, the number of melanoma cell lines that are suitable for the above studies is limited; furthermore, only few cell lines have been characterized for genetic alterations in detail using different molecular cytogenetic approaches (4). Genetic instability of tumor cells leads to the development of several clones with diverse metastatic potential (ranging from non-metastatic to highly metastatic subclones). The natural course of melanoma progression involves distant metastasis formation

into different organs, most frequently the lungs, liver and bones. To analyze the molecular aspects of this metastatic process, we established a new melanoma cell line, designated M35/01, arising from a primary superficial spreading melanoma. The advantage of this novel cell line is that, similarly to the original tumor, it metastasizes to the liver after injecting into SCID-mice, therefore it might be a powerful model for the investigation of molecular alterations associated with organ-specific metastasis.

The new primary melanoma cell line M35/01 was characterized by G-banding, chromosomal and array comparative genomic hybridization (cCGH and aCGH), spectral karyotyping (SKY-FISH), and fluorescence *in situ* hybridization (FISH) using centromeric-, locus-, and gene-specific, as well as by chromosome painting probes. In addition, we had sufficient amount of DNA from the patient's primary tumor for cCGH to compare the genetic patterns of the cell line with that of the original tumor. Several common alterations were present both in the primary tumor and in the derived cell line, and a few additional changes were found only in M35/01. The spectral karyotype of M35/01 revealed numerous structural alterations and aCGH analysis showed many chromosomal abnormalities.

3. MATERIALS AND METHODS

3.1. Establishment of the M35/01 cell line

Tissue sample for cell culturing was obtained from the vertical growth phase of a primary melanoma surgically removed from a 69-year old male patient diagnosed with superficial spreading melanoma in 2001. The freshly resected tumor tissue was minced into small pieces and digested for 3-4 hours at 37°C in RPMI 1640 medium containing 0.1% collagenase type I, 0.01% hyaluronidase type IV, 0.01% DNAse type I, 100 U/ml penicillin, 100 µg/ml streptomycin (all from Sigma, St. Louis, MO), and 50 µg/ml gentamicin (Gibco BRL, Life Technologies, Paisley, Scotland). The resulting mixture was filtered through four layers of sterile gauze, washed and resuspended in RPMI 1640 supplemented with 10% fetal bovine serum (Sigma) and antibiotics. After overnight incubation at 37°C in a 5% CO_2 atmosphere, non-adherent cells were discarded and adherent melanoma cells were cultured to develop a continuously growing cell culture. The growth rate and the expression of melanoma markers were checked regularly. A consistent positive staining with HMB45 antibody was observed, while MART-1 expression was lower, and S100B protein could not be detected.

3.2. Chromosome preparation and G-banding

Cell culture was harvested according to standard cytogenetic techniques. Briefly, M35/01 cells were arrested with 0,01 µg/ml colcemid (Gibco BRL) for 2,5 hour. After hypotonic treatment (0.075 M KCl, 20 min), cells were fixed in methanol/acetic acid (3:1). Cell suspensions were dropped on cold, water-rinsed slides and air-dried at room temperature. G-banding was performed using standard protocol.

3.3. Fluorescence *in situ* hybridization

FISH was carried out as described earlier (5, 6) using directly labeled locus-specific probes for 1p36, *EGR*-1 (Qbiogene GmbH, Heidelberg, Germany), *EGF-R*, *CYCLIN-D1*, 9p21, *RB1* (Vysis Inc. Downers Grove, IL, USA), and indirectly labeled centromere-specific probes for chromosomes 1-13, 15-18, 20-22 and X and Y. Centromere-specific probes were isolated from bacteria clones generously supplied by Resource for Molecular Cytogenetics (University of California Cancer Center, San Francisco, CA, USA) and Molecular Genetic Resources (University of Bari, Bari, Italy). Isolated DNAs were labelled with biotin-dUTP and digoxigenin-dUTP by nick translation using BioNick kit according to the protocol of the supplier (Gibco BRL). Painting probes for chromosomes 2, 4-7, 11, 13, 15, 20-22 and 6p21 band were obtained from Qbiogene and Vysis.

Samples were scored for the number of fluorescent signals in each nucleus by using a fluorescence microscope (OPTON, Oberkochen, Germany) equipped with selective filters for the detection of FITC, SpectrumGreen, SpectrumOrange and DAPI. Approximately 200-500 nuclei and/or 10 metaphases were scored for each specimen. Three-color images were captured using a digital imaging analysis system (ISIS, Metasystems GmbH, Althussheim, Germany) (6).

3.4. Spectral karyotyping

Spectral karyotyping was performed as described by Schröck *et al.* (7). Briefly, chromosome-specific libraries generated by PCR from flow-sorted human chromosomes were directly labeled with nucleotides conjugated to five different dyes (FITC, Rhodamine, Texas Red, Cy5 and Cy5.5). All 24 chromosome libraries were hybridized simultaneously to the metaphases. After washing, the slides were stained with DAPI in antifade medium. The different spectra were distinguished using the SD300 spectral bio-imaging system (Applied Spectral Imaging Ltd, Migdal Ha'emeg, Israel). This system allows the measurement of the full visible light spectrum at each pixel of the image by using a Sagnac interferometer. A classification algorithm was used to differentiate between different spectra in the image and also, to assign pseudocolors to all the pixels, with similar spectral characteristics. The DAPI image was captured separately and inverted to give a G-banding pattern. The chromosomes were then sorted automatically into a karyotype table.

3.5. Chromosomal comparative genomic hybridization

CGH was performed as described earlier in detail (8). Briefly, tumor cell DNA and normal reference DNA were extracted using phenol-chloroform-isoamylalcohol (PCI) extraction and labeled by standard nick translation with SpectrumGreen-5-dUTP and SpectrumRed-5-dUTP, respectively (Vysis Inc.). Labeled DNAs (200 ng each) and 20 µg of unlabeled Cot-1 DNA (Gibco BRL) were hybridized onto commercially available normal metaphase chromosomes (Vysis Inc.). Hybridizations were evaluated using a digital image analysis system (ISIS, Metasystems GmbH).

3.6. Array comparative genomic hybridization

For aCGH analysis, microarrays containing 2460 overlapping BAC clones with an average resolution of 1.4 Mb were used. BAC clones were prepared by ligation mediated PCR as described by Snijders *et al* (9). DNA

clones were robotically spotted in triplicate onto chromium-coated slides (Nanofilm, Westlake Village, CA), followed by UV cross linking. Hybridization and scanning were carried out in the Microarray Core at the Comprehensive Cancer Center, University of California, San Francisco. Array hybridization was performed as described by Pinkel *et al.* (10). The imaging setup and costume software were described by Pinkel *et al.* (10). Image analysis was performed using the SPOT version 1.2 and SPROC version 1.1 software packages (11). Log_2 ratios of chromosomal gains and losses were listed by an algorithm using a flexible symmetrical threshold which was based on the standard deviation of the data sets of the sample. Array validation was performed using normal human female reference DNA (12). Array spots with higher than 0.55 Log_2 ratio were considered as gains and values less than -0.55 as losses.

4. RESULTS

In the present study, complex chromosomal alterations have been determined in detail for a recently established melanoma cell line designated M35/01. Cells from passages 38-42 were used for chromosome preparation. By G-banding, the number of chromosomes per cell varied between 65 and 82 reflecting a nearly triploid karyotype with several chromosomal rearrangements. However, this karyotype was incomplete and seemed to be very complex, with several chromosomes to which materials of unknown origin had been added. Standard G-banding turned out to be obviously insufficient to obtain a reliable and reasonably complete pattern of the genomic changes.

4.1. Detection of structural alterations using painting probes and SKY-FISH analysis

By G-banding, several chromosomal translocations could be seen but due to the low resolution of this technique their origins remained mainly unknown. To resolve this problem, we hybridized painting probes specific to chromosomes 2, 4-7, 11, 13, 15 and 20-22 on metaphase spreads. The most complex rearrangement could be found for chromosomes 2, 7, 15 and 20. With our probe combinations, only a translocation between chromosome 13 and 21 could be confirmed, for the other chromosomes the recipient chromosomal part remained unknown.

In order to clarify the uncertain structural alterations, we performed SKY-FISH experiment, the results being shown in Figure 1. SKY-FISH analysis confirmed some of the observed alterations seen by G-banding and FISH, i.e. most of the chromosomes were present in 3 or 4 copies/cell. Besides numerical alterations, SKY-FISH analysis revealed several structural aberrations with at least 15 different rearrangements involving 12 chromosomes. Complex rearrangements could be observed between chromosomes 2 and 15 [der(2)t(2;15)], 2 and 12 [der(2)t(2;12;2)], 13, 21 and Y [der(13)t(Y;13;21)], 6 and 15 [der(15)t(6;15)], 7 and 17 [der(17)t(7;17)], and chromosome 19 and 22 [der(19)t(19;22;19)]. Marker chromosomes could be detected neither by banding analyses nor by SKY-FISH. The final karyotype by SKY-FISH was as follows: 68,XXY, 3Xder(2)t(2;15), -4, +5, +7, -9, -10, der(2;12;2), +der(13)t(Y;13;21), 2Xder(15)t(6;15), -16, der(17)t(7;17), -18, der(19)t(19;22;19), add(20), +add(20), -21, -22.

To further clarify the specific genetic alterations commonly altered in melanoma and other cancers, we applied gene-specific probes for *EGR1* (5q31), *EGFR* (7p12), *CYCLIN-D1* (11q13), *p16* (9p21), *C-MYC* (8q24) and *RB1* (13q14). As a result of double target hybridizations (i.e. combined FISH with a gene-specific probe and a centromeric probe of the chromosome where the gene is located) we observed similar distribution pattern for both centromeric and gene specific probes, suggesting that none of these genes were amplified or deleted in relation to the centromeres.

4.2. Detection of numerical aberrations in interphase nuclei

To define DNA ploidy at chromosomal level, we performed interphase FISH analysis using centromere-specific probes for almost all chromosomes. Because of the high homology of chromosome 14 and 19 centromeric regions, we did not investigate these alterations. In case of chromosomes 13 and 21, probes specific to 13q14 and 21q22.13-q22.2 regions were used.

Table 1 shows the distribution of chromosome centromeric copy numbers per cell in the M35/01 cell line. For chromosomes 1, 3, 4, 6, 8, 11 and 21 three centromere-specific signals could be detected in more than 50% of the cells. The major cell population (> 60% of the cells) for chromosomes 2, 5, 7, 13, 15 and 17 was tetrasomic. Chromosome 12 exhibited three copies in 42% and four centromeric signals could be seen in 48% of the cells. Among autosomes, chromosomes 9, 10, 16, 18, 20 and 22 had a major population with two copies, but the frequency of the disomic cells was above 90% only for chromosome 18. For chromosome 20 and 22, high number of trisomic cells (31% and 21%, respectively) was also present. The cell line was highly heterogenic for chromosome 9 showing two copies in 44%, three copies in 16% and four copies in 18% of the cells. Both sex chromosomes were duplicated, the dominant clone was definitely disomic (98% for chromosome X and 94% for chromosome Y).

4.3. Genetic differences between M35/01 cell line and the original primary tumor

To compare the genetic alterations between the original tumor and the M35/01 cell line, cCGH analysis was performed on both samples. The results are shown in Table 2. Besides several common alterations, we found few chromosomal changes appearing only in the established cell line. Alterations present in both the original primary tumor and the new cell line included losses of chromosomes 9 and 10, 16q and 17p, and gains of 6p22-pter, 7q22-qter and 15q. Deletion of chromosome 4, a larger segment of chromosome 12, 16p and the loss of the entire chromosome 18, as well as gain of chromosome 20p12-pter, were detected only in the cell line.

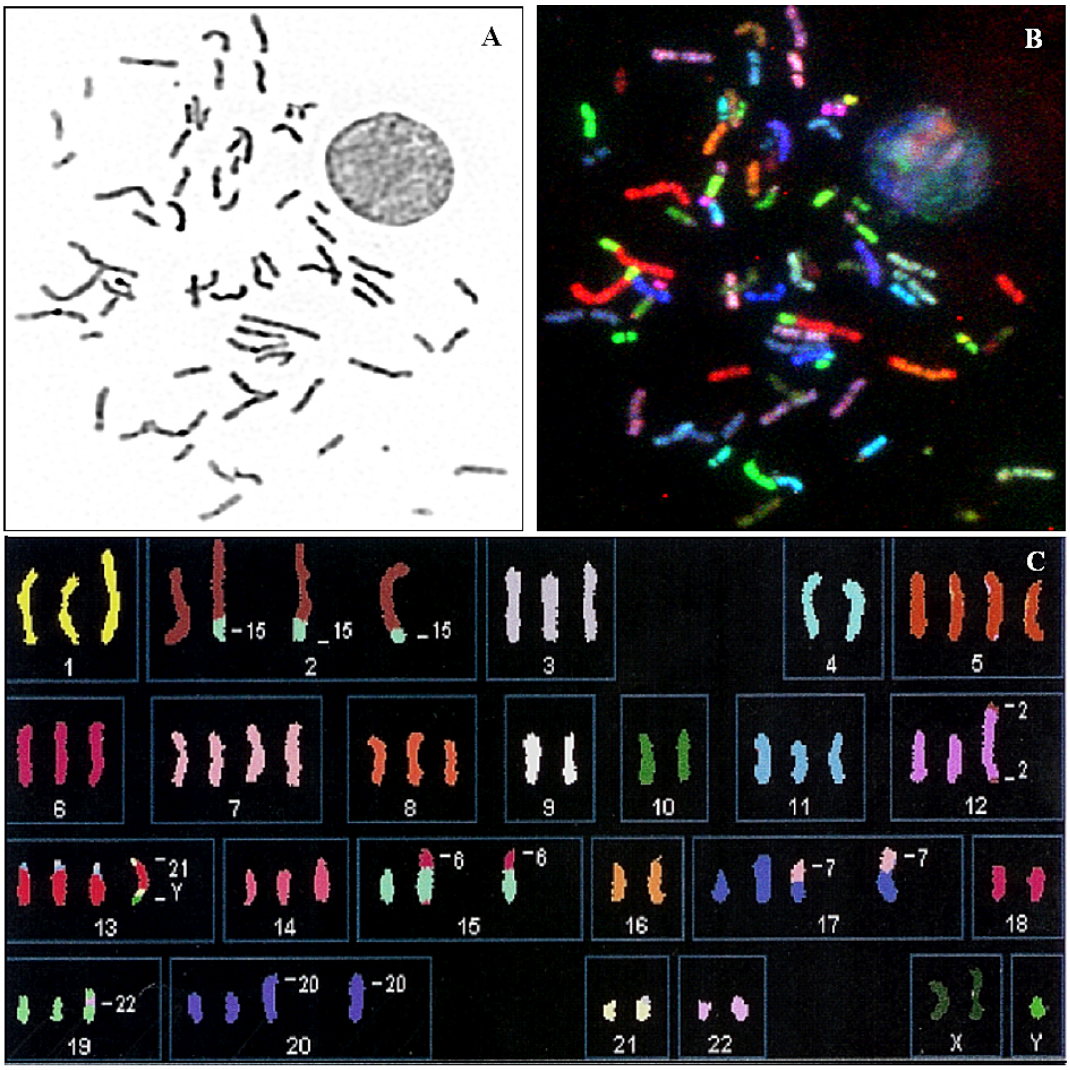

Figure 1. Spectral karyotyping of the M35/01 melanoma cell line. A) A representative metaphase spread shown as an inverted DAPI image. B) SKY spectral image of chromosomes. C) Spectral classification of melanoma cell line M35/01 (each chromosome was assigned a pseudo color according to its measured spectrum). Numbers next to the chromosomes indicate the origins of the translocated chromosome fragments. Complex rearrangements could be observed between chromosomes 2 and 15 [der(2)t(2;15)], 2 and 12 [der(2)t(2;12;2)], 13, 21 and Y [der(13)t(Y;13;21)], 6 and 15 [der(15)t(6;15)], 7 and 17 [der(17)t(7;17)], and chromosomes 19 and 22 [der(19)t(19;22;19)].

To further define the regions involved in amplifications and deletions in the M35/01 cell line, aCGH analysis was performed on microarray containing 2460 well characterized BAC clones. Overview of chromosomal gains and losses as detected by array CGH are shown in Figure 2. The number of gained sequences were 226 (log_2 ratio >0.55) and deletion was found on 281 BAC clones (log_2 ratio < 0.55). Genomic regions showing high-level amplifications (defined as log_2 ratio >1) were identified on 15q15 (log_2 ratio: 1.02), 15q21-q25 (log_2 ratio: 1.31-1.14), 7q22-q31.1 (log_2 ratio: 1.1) and 20q13 (log_2 ratio: 1.1). Individual significantly deleted clones included 9q21 (log_2 ratio: - 0.86), 4q12-q33 (log_2 ratio: -(0.85-0.69), 18p11.21-23 (log_2 ratio: - 0.84), 18q11-12 (log_2 ratio: - 0.73), 18q21-23(log_2 ratio: -(0.81–0.7), 12q24.33 (log_2 ratio: - 0.84), 17p12 (log_2 ratio: - 0.78), 9p21 (log_2 ratio: - 0.71), 16q21 (log_2 ratio: - 0.74), 16q23 (log_2 ratio: - 0.69), 16q24 (log_2 ratio: - 0.69). Chromosome alterations of 6p and 15q as detected by both CGH techniques are displayed in Figure 3 (chromosome 6) and Figure 4 (chromosome 15). In addition to chromosomal and array CGH profiles of the new M35/01 cell line for these two chromosomes, the cCGH profile of the original primary melanoma is displayed. Alterations found by conventional CGH were supported by aCGH with a much higher resolution.

Comparison of SKY-FISH and CGH profiles of selected chromosomes allowed us to define not only the copy number increases of a certain chromosome but also the identification of the translocated parts. The partial

Table 1. Centromeric copy number distribution in M35/01 cell line studied by interphase FISH [1]

Chromosomes	Signals/Cell					
	0-1	**2**	**3**	**4**	**5**	**>6**
Chr 1	0	13	**70** [2]	17	0	0
Chr 2	0	0	6	**92**	1	0
Chr 3	0	6	**66**	22	6	0
Chr 4	0	19	**78**	2	1	0
Chr 5	0	2	10	**79**	9	0
Chr 6	0	11	**89**	0	0	0
Chr 7	0	6	22	**66**	3	3
Chr 8	0	7	**85**	6	1	1
Chr 9	8	**44**	16	18	6	8
Chr 10	6	**88**	10	1	0	1
Chr 11	0	6	**82**	9	2	1
Chr 12	0	4	**42**	**48**	1	5
Chr 13	0	3	17	**73**	4	3
Chr 15	0	2	12	**81**	3	2
Chr 16	0	**80**	10	9	1	0
Chr 17	0	1	6	**90**	3	0
Chr 18	0	**94**	0	6	0	0
Chr 20	1	**64**	31	3	1	0
Chr 21	0	27	**67**	5	1	0
Chr 22	1	**75**	21	2	1	0
Chr X	0	**98**	1	1	0	0
Chr Y	4	**94**	2	0	0	0

[1] For all experiments, peripheral mononuclear cells were used as controls. [2] Dominant cell populations are labeled with bold

Table 2. Summary of chromosome copy number gains and losses in the original primary melanoma and in the novel melanoma cell line 35/01 as detected by cCGH

Tissue sample	**Losses**	**Gains**
Primary tumor	9p, 9q, 10p, 10q, 16q, 17p	6p21.3-pter, 7q22-qter, 15q
M35/01 cell line	**4**[1], 9p, 9q,, 10p, 10q, **12p12-q13.12**, **16p**, 16q, 17p, **18**	6p22-pter, 7q22-qter, 15q, **20p12-qter**

[1] Losses and gains present only in the derived cell line are labeled with bold

SKY-FISH and CGH profiles of chromosomes 2, 15, 6, 7 and 17 are shown in Figure 5A (SKY-FISH) and Figure 5B (chromosomal CGH). CGH ratio profile for chromosome 2 indicates normal pattern. However, SKY-FISH clearly shows translocated segments from chromosome 15q. The origin of the translocated part on chromosome 15 is the result of structural rearrangement of chromosome 6p. Similarly, extra chromosome 7 segments on chromosome 17 originated from 7q22-qter (aCGH $\log_2$ ratio > 0.55).

5. DISCUSSION

Cell lines are useful tools in understanding the molecular genetic background of different biological behavior of malignancies, and in many types of cancers, they represent the most readily available models for cancer research. Recently, we have successfully established a novel melanoma cell line (M35/01) from a metastatic superficial spreading malignant melanoma. The present study is aimed at characterizing the genetic alterations of this cell line using a series of molecular cytogenetic techniques. Similarly to the original tumor, this new melanoma cell line metastatised to the liver in an animal model system. Using G-banding and various *in situ* hybridization techniques, such as interphase- and SKY-FISH, chromosomal and array CGH, among melanoma-specific chromosomal changes, we observed new genetic alterations rarely seen before.

By chromosome banding, the number of chromosomes varied between 65 and 82, reflecting a nearly triploid karyotype with several chromosomal rearrangements. However, the karyotype obtained was incomplete for different chromosomes to which materials of unknown origin had been added. Standard G-banding was obviously insufficient to obtain a reliable and reasonably complete pattern of chromosomal changes. Fluorescence *in situ* hybridization on interphase cells and metaphase chromosomes were performed to further define the numerical and structural alterations. Centromere-specific probes were used for all chromosomes (except 14 and 19). Chromosomes 2, 5, 7, 13, 15, and 17 were tetrasomic, chromosomes 1, 3, 4, 6, 8, 11 and 21 turned out to be trisomic, while chromosomes 10, 16, 18, 20, X and Y proved to be disomic in more than 50% of the cells. Based on the FISH data, we assume that the genome had undergone a global duplication first, than subsequent losses of the genome occurred during tumor progression. As it was shown, tetraploidization is an important milestone in the clonal evolution of many solid tumors (13).

Using SKY-FISH analysis, we observed that numerical alterations were associated with structural rearrangements, affecting chromosomes 2 and 15; 2 and 12; 13, 21 and Y; 15 and 6; 7 and 17; 19 and 21. The rearrangement between chromosomes 6 and 15 was also seen in melanoma cell lines by others (14, 15). The numerical alterations of chromosomes in the established cell line were representative of those ones altered in a number of metastatic melanomas that were analysed by standard cytogenetic method earlier (16).

By chromosomal CGH we could compare the genetic pattern of the native tumor and the developed cell line. Comparing the CGH profiles, we observed that the majority of copy number alterations were identical in both, indicating that the cell line retained the genetic characteristics of the parental tumor. Common chromosomal alterations included gains on chromosomal regions 6p22-pter, 7q22-qter, 15q15-qter, and losses on 9, 10, 16q and17p. Deletions of chromosome 4, 12p12-q13.12, 16p and 18, as well as gains on chromosome 20 could be observed only in the cell line. This divergence can be explained in different ways: *i.)* the cell line, similarly to the parental tumor, is heterogeneous, but the ratio of cells exhibiting the observed different alterations is higher in the M35/01 cell line, *ii.)* the normal cell contamination of the primary melanoma can mask some alterations present in a small fraction of cells, thus it may cover less dominant changes in a genetically heterogeneous tumor (17), *iii.)* it can not be excluded either that alterations seen only in the cell line are the results of *in vitro* cell culturing.

Array CGH analysis of the cell line showed similar results to those obtained by conventional CGH analysis,

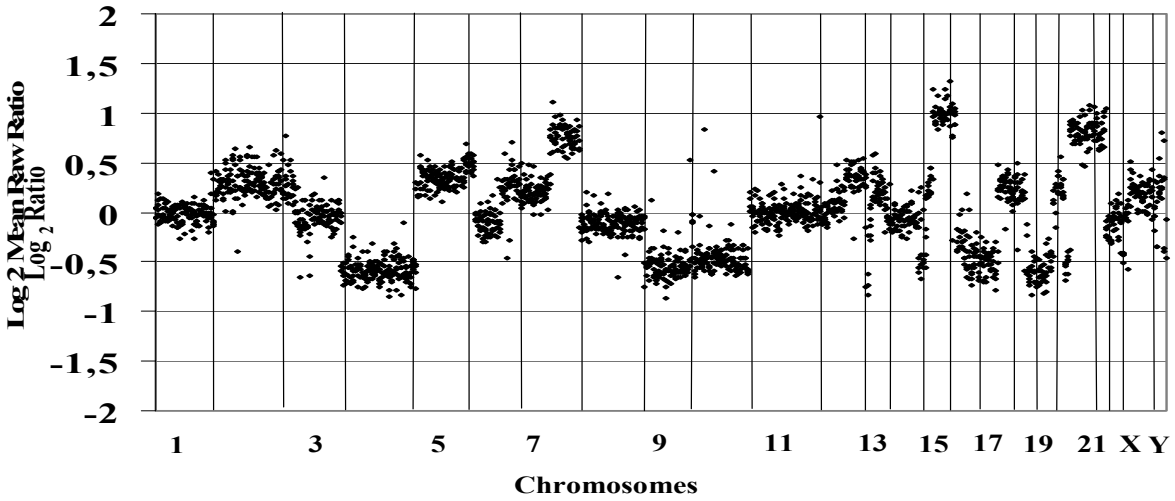

Figure 2. Array based comparative genomic hybridization of the M35/01 melanoma cell line. Log_2 ratios along the genome illustrate genomic imbalances (raw data are presented). The significant gains of chromosomal regions 6p22-pter, 7q21-qter, 15q15-qter and chromosome 20, as well as losses of chromosomes 4, 9, 10, 16 and 18 and chromosomal regions 5p14-pter, 14q32-qter seen by conventional CGH were also detected by array CGH.

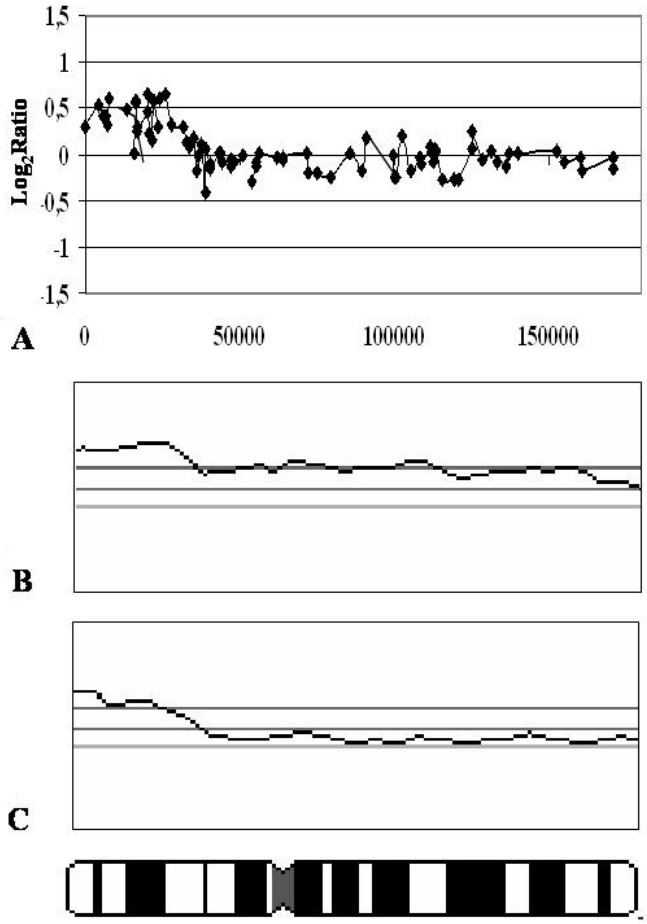

Figure 3. Comparison of array CGH and chromosomal CGH alterations of chromosome 6. A) Array and B) conventional CGH profiles for chromosome 6 exhibit similar patterns of the derived M35/01 melanoma cell line. C) Chromosomal CGH ratio profile of chromosome 6 of the original tumor is similar to that of the derived cell line.

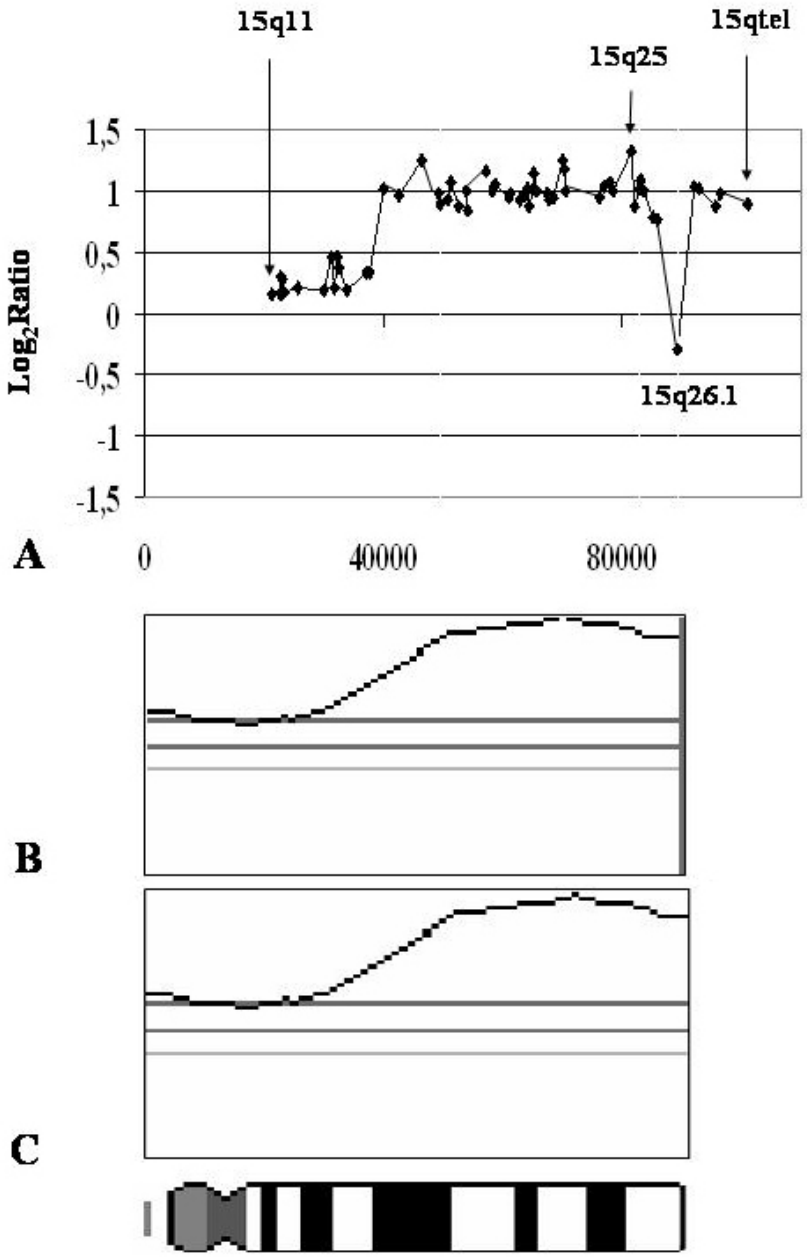

Figure 4. Comparison of the alterations of chromosome 15 detected by array and chromosomal CGH. A) Array and B) conventional CGH profiles for chromosome 15 of the M35/01 melanoma cell line exhibit similar patterns. The highest ratio value (indicating by an arrow) was detected on 15q21 (Log_2 ratio = 1.16). C) Chromosomal CGH ratio profile of chromosome 15 of the original tumor is similar to that of the derived cell line. Deletion of the 15q26.1 DNA segment was only seen by array CGH.

representing regions that are frequently altered in melanoma, but new alterations were also found. Out of the 73 clones representing chromosome 15, the log_2 ratio was above 1.00 in 13 clones. The highest ratio value was detected on chromosome 15q25 (log_2 ratio = 1.31), meaning approximately 3-5 signals on this locus; by chromosomal CGH, the overrepresentation of the entire chromosome 15 could be observed, no such an alteration was reported earlier. Relatively high amplification was observed on the 15q21-qter region. Genes on this region that might be involved in melanomagenesis include thrombospondin-1 (*TSP1*), the product of which cooperates with the extracellular matrix and plays a role in several biological processes. In melanoma, its altered expression in the stroma is related to poor prognosis (18). The overrepresented regions of chromosome 15 is associated with unbalanced translocations involving chromosomes 2 and 6. Based on the available CGH pattern of chromosome 6p, it is possible that the translocated parts of chromosome 15 originates from the 15q25-q26 locus.

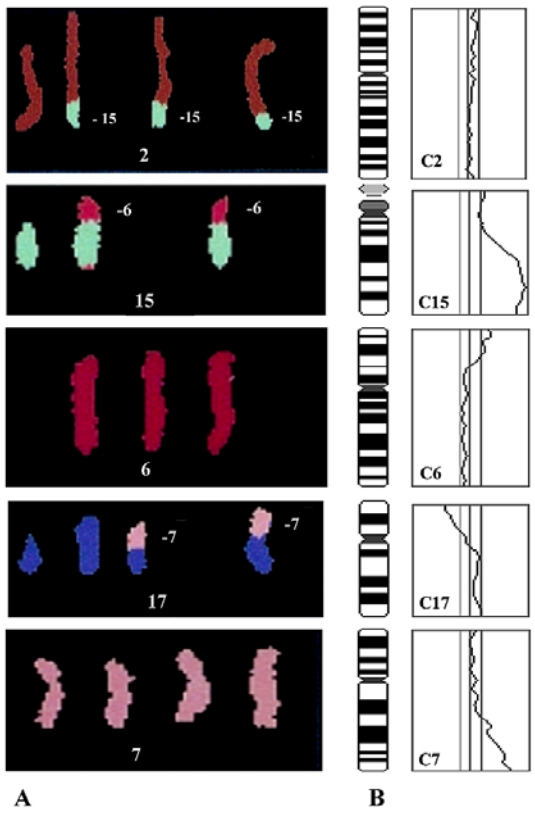

Figure 5. CGH and SKY-FISH images of selected chromosomes. A) Partial SKY-FISH-karyotypes for chromosomes 2, 15, 6, 17 and 7 show special translocations including 3Xder(2)t(2;15), 2Xder(15)t(6;15) and 2Xder(17)t(7;17). B) CGH ratio profile for chromosome 2 indicates normal pattern, high level gain for 15q, gain for the 6p21-pter, deletion for 17pter, and gain for 7q32-qter regions, respectively.

Chromosome 6 plays a special role in melanoma progression and it is presumed that both tumor suppressor genes and oncogenes may be present on this chromosome (19). The latter is supported by frequent amplification of chromosome 6p, although no gene related to melanoma pathogenesis could be detected so far on this arm. Amplification of the short arm as well as deletion of the long arm of chromosome 6 have been reported both in native tissue samples and cell lines. By standard cytogenetics, and also by CGH analysis, 6p amplification is often associated with 6q deletion and probably it is related to isochromosome formation (16). Among the 47 BAC clones of chromosome 6 on the array, nine showed amplification, five of which were at 6p22, two at 6p22-p23, and one at 6p24-p25. Melanoma-specific genes on these particular loci have not been described yet. None of the CGH analyses showed deletion on this chromosome arm.

Aberrations of chromosome 7 are often related to poor prognosis in melanoma, and this is supposed to be a late event (16). Several amplified loci could be detected, by array CGH on chromosome 7q, mainly on the 7q31-q36 region. This region harbours the *BRAF* oncogene, whose mutation can be found in about two-thirds of malignant melanomas, while it is absent in uveal melanoma (20). Besides the numerical aberrations of chromosome 7, we detected a translocation between chromosomes 7 and 17.

The most often studied and the best known locus in melanoma progression is 9p21 (21, 22). Cyclin-dependent kinase 2A (*P16, CDKN2, INK4, MTS1*) and 2B (*INK4B, MTS2, P15)* genes are located in this region (23). By chromosomal CGH, the entire chromosome 9 was deleted, however, by array CGH the most affected region was 9q. The loss of whole chromosome 9 as an early event in tumorigenesis was described by Bastian *et al.* (19), and supported by data on the frequent loss of this chromosome in dysplastic naevi (24).

The other frequently lost chromosome in melanoma is chromosome 10, which, similarly to the loss of chromosome 9, occurs early during melanoma development. Loss of heterozygozity (LOH) studies found 10q deletion in 30-50% of primary melanomas, mainly in thinner lesions (25). The *PTEN/MMAC1* gene on 10q23.3 was discovered as a tumor suppressor gene showing mutations in a high number of tumors. In more than 40% of melanoma metastases, the gene could not be detected or it was mutated (26). Damaged function of *PTEN/MMAC1* was also described in gliomas, another type of cancer with neural crest origin (27). *PTEN/MMAC1* might play a role in later stages of melanoma progression.

Bastian *et al.* compared genetic alterations by CGH and showed that in the superficial spreading and lentigo maligna subtypes, which develop most frequently on sun-exposed sites, alterations of 13q and 17p are more frequent (28). Deletion of 17p was among the common alterations and it could be related to the mutation of p53 gene, which plays an essential role in DNA-repair. Another complex chromosomal rearrangement in the M35/01 cell line included chromosomes 13, 21 and Y. Chromosome 13 has not appeared in melanoma literature as one of the possibly affected chromosomes in the progression of this malignancy, albeit the results of multicolor-FISH published by Schulten *et al.* show complex rearrangements including chromosome 13 in four out of the seven analyzed melanoma cell lines (4). Probably, the involvement of chromosome 13 is just a casual event which is supported by the fact that in the four above mentioned cell lines the translocation partner of chromosome 13 is different in all cases. However, these findings draw attention to chromosome 13 as an often rearranged chromosome in melanoma.

Copy number changes on chromosome 16 have been reported in connection with melanoma. In normal human skin, E-cadherin (16q22) is expressed on the surface of all cells of the epidermis (keratinocytes, melanocytes, Langerhans-cells); P-cadherin can be found only on keratinocytes in basal layer; and N-cadherin is present only on fibroblasts and endothelial cells. Herlyn *et al.* emphasized cadherin expression shift during melanoma development (29) and it was hypothesized that escape of melanoma cells from E-cadherin-regulated control of

keratinocytes might be a key event in melanoma pathogenesis.

A small segment of chromosome 22 was inserted into one copy of chromosome 19 but the role of this event is unclear in melanoma progression. Both these chromosomes contain several known genes with possible function in tumorigenesis, e.g. lamin-B2 (19p13), which is critical in the maintenance of nuclear stability and chromatin structure; or a gene encoding a zinc-finger protein with transcriptional functions (19q13). Translocation of chromosome 22 to 19p13 may destroy normal gene function, thus leading to the destabilization of the nucleus and loosening chromatin structure.

The alteration observed on chromosome 20 may be the result of isochromosome formation. Unfortunately, chromosome arm specific painting probes were not available for this study. One of the candidate genes in melanoma development on chromosome 20 is matrix metalloproteinase-9 (MMP-9, 20q11-q13). Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in metastasis formation (30).

In conclusion, we report comprehensive genetic analyses of a newly established melanoma cell line by a combination of molecular cytogenetic techniques, including G-banding, FISH, SKY-FISH, chromosomal and array CGH. Using these methods, we described a reservoir of common and new genetic alterations. We could compare the genomic aberrations of the cell line to the original primary tumor by CGH and found that most of the alterations were common in both, indicating that the cell line retained the genetic characteristics of the parental tumor. The novel melanoma cell line, similarly to the original superficial spreading primary melanoma, was metastatic to the liver and it might be a suitable model for *in vitro* studies of this melanoma subtype.

6. ACKNOWLEDGEMENT

We thank Katalin Derecskei for her precious laboratory assistance. This work was supported by the Hungarian Ministry of Education, Hungarian Scientific Research Fund (OTKA T 04875), National Research and Development Program Hungary (NKFP-1B-0013/2002), Hungarian Ministry of Health (ETT 376/2003) and UD MHSC Mecenatura 0017/2005.

7. REFERENCES

1.Camplejohn, R.S.: DNA damage and repair in melanoma and non-melanoma skin cancer. *Cancer Surv* 26,193–206 (1996)

2. Molife, R. & B. W. Hancock BW: Adjuvant therapy of malignant melanoma. *Crit Rev Oncol Hematol* 44(1),81-102 (2002)

3. Meric, J.B., O. Rixe & D. Khayat: Metastatic malignant melanoma. *Drugs Today (Barc)* 39 Suppl C,17-38 (2003)

4. Schulten, H.J., B. Gunawan, F. Otto, R. Hassmann, C. Hallermann, A. Noebel & L. Fuzesi: Cytogenetic characterization of complex karyotypes in seven established melanoma cell lines by multiplex fluorescence *in situ* hybridization and DAPI banding. *Cancer Genet Cytogenet* 133(2),134-141 (2002)

5. Balázs, M., K. Matsumura, D. Moore, D. Pinkel, J.W. Gray & F. Waldman: Karyotypic heterogeneity and its relation to labeling index in interphase breast tumor cells. *Cytometry* 20,62-73 (1995)

6. Treszl, A., R. Ádány, Zs. Rákosy, L. Kardos, Á. Bégány, K. Gilde & M. Balázs: Extra copies of c-myc are more pronounced in nodular melanomas than in superficial spreading melanomas as revealed by fluorescence in situ hybridization. *Cytometry* 60(B),37-46 (2004)

7. Schröck, E., S. du Manoir, T. Veldman, B. Schoell., J. Wienberg J., M.A. Ferguson-Smith, Y. Ning, D.H. Ledbetter, I. Bar-Am, D. Soenksen, Y. Garini & T. Ried: Multicolor Spectral Karyotyping of Human chromosomes. *Science* 273,494-497 (1996)

8. Balázs, M., Zs. Ádám, A. Treszl, Á. Bégány, J. Hunyadi & R. Ádány: Chromosomal imbalances in primary and metastatic melanomas revealed by comparative genomic hybridization. Cytometry 46,222-232 (2001)

9. Snijders A.M., N. Nowak, R. Segraves, S. Blackwood, N. Brown, J. Conroy, G. Hamilton, A.K. Hindle, B. Huey, K. Kimura, S. Law, K. Myambo, J. Palmer, B. Ylstra, J.P. Yue, J.W. Gray, A.N. Jain, D. Pinkel & D.G. Albertson: Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* 29(3),263-264 (2001)

10. Pinkel, D., R. Segraves, D. Sudar, S. Clark, I. Poole, D. Kowbel, C. Collins, W.L. Kuo, C. Chen, Y. Zhai, S.H. Dairkee, B.M. Ljung, J.W. Gray & D.G. Albertson: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 20(2),207-211 (1998)

11. Jain, E.: Distributed computing in bioinformatics. *Appl Bioinformatics* 1(1),13-20 (2002)

12. Paris, P.L., D.G. Albertson, J.C. Alers, A. Andaya, P. Carroll, J. Fridlyand, A.N. Jain, S. Kamkar, D. Kowbel, P.J. Krijtenburg, D. Pinkel, F.H. Schroder, K.J. Vissers, V.J. Watson, M.F. Wildhagen, C. Collins & H. Van Dekken: High-resolution analysis of paraffin-embedded and formalin-fixed prostate tumors using comparative genomic hybridization to genomic microarrays. *Am J Pathol* 162(3),763-770 (2003)

13. Shackney, S.E., C.A. Smith, B.W. Miller, D.R. Burholt, K. Murtha, H.R. Giles, D.M. Ketterer & A.A. Pollice: Model for the genetic evolution of human solid tumors. *Cancer Res* 15;49(12),3344-3354 (1989)

14. Guan, X.Y., H.E. Zhang, H. Zhou, J.S. Sham, J.M. Fung & J.M. Trent: Characterization of a complex chromosome rearrangement involving 6q in a melanoma cell line by chromosome microdissection. *Cancer Genet Cytogenet* 134(1),65-70 (2002)

15. Wiltshire R.N., T.R. Dennis, V.K. Sondak, P.S. Meltzer & J.M. Trent: Application of molecular cytogenetic techniques in a case study of human cutaneous metastatic melanoma. *Cancer Genet Cytogenet* 31(2),97-103 (2001)

16. Trent J.M., F.L. Meyskens, S.E. Salmon, K. Ryschon, S.P.L. Leong, J.R. Davis & D.L. McGee: Relation of cytogenetic abnormalities and clinical outcome in metastatic melanoma. *N Eng J Med* 322,1508-1511 (1990)
17. Isola, J., S. DeVries, L. Chu, S. Ghazvini & F. Waldman: Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. *Am J Pathol* 145(6),1301-1308 (1994)
18. Trotter M.J., R. Colwell & V.A. Tron: Thrombospondin-1 and cutaneous melanoma. *J Cutan Med Surg* 7(2),136-141 (2003)
19. Bastian, B.C., P.E. LeBoit, H. Hamm, E.B. Brocker & D. Pinkel: Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. *Cancer Res* 58(10),2170-2175 (1998)
20. Edmunds, S.C., I.A. Cree, F. Di Nicolantonio, J.L. Hungerford, J.S. Hurren & D.P. Kelsell: Absence of BRAF gene mutations in uveal melanomas in contrast to cutaneous melanomas. *Brit J Cancer* 88,1403-1405 (2003)
21. Box, N.F., D.L. Duffy, W. Chen, M. Stark, N.G. Martin, R.A. Sturm & N.K. Hayward: MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. *Am. J. Hum. Genet* 69,765-773 (2001)
22. Sharpless N.E., S. Alson, S. Chan, D.P. Silver, D.H. Castrillon & R.A. DePinho: p16INK4a and p53 deficiency cooperate in tumorigenesis. *Cancer Res* 62,2761-2765 (2002)
23. Petty, E.M., J.L. Bolognia, A.E. Bale & T. Yang-Feng: Cutaneous malignant melanoma and atypical moles associated with a constitutional rearrangement of chromosomes 5 and 9. *Am J Med Genet* 45,77-80 (1993)
24. Cowan, J.M., R. Halaban & U. Francke: Cytogenetic analysis of melanocytes from premalignant nevi and melanomas. *J Natl Cancer Inst* 80(14),1159-1164 (1988)
25. Poetsch, M, T. Dittberner & C. Woenckhaus: Can different genetic changes characterize histogenetic subtypes and biologic behavior in sporadic malignant melanoma of the skin? *Cell Mol Life Sci* 60(9),1923-1932 (2003)
26. Zhou Y.H., F. Tan, K.R. Hess & W.K. Yung: The expression of PAX6, PTEN, vascular endothelial growth factor, and epidermal growth factor receptor in gliomas: relationship to tumor grade and survival. *Clin Cancer Res* 9(9),3369-3375 (2003)
27. Tomlinson I.P., N.E. Beck & W.F. Bodmer: Allele loss on chromosome 11q and microsatellite instability in malignant melanoma. *Eur J Cancer* 32A(10),1797-802 (1996)
28. Bastian, B.C., A.B. Olshen, P.E. LeBoit & D. Pinkel: Classifying melanocytic tumors based on DNA copy number changes. *Am J Pathol* 163(5),1765-1770 (2003)
29. Herlyn, M., C. Berking, G. Li & K. Satyamoorthy: Lessons from melanocyte development for understanding the biological events in naevus and melanoma formation. *Melanoma Res* 10(4),303-312 (2000)
30. Barks, J.H., F.H. Thompson, R. Taetle, J.M. Yang, J.F. Stone, J.A. Wymer, R. Khavari, X.Y. Guan, J.M. Trent, D. Pinkel & M.A. Nelson: Increased chromosome 20 copy number detected by fluorescence *in situ* hybridization (FISH) in malignant melanoma. *Genes Chromosomes Cancer* 19(4),278-85 (1997)

Abbreviations: FISH: fluorescence *in situ* hybridization; aCGH: array comparative genomic hybridization; cCGH: chromosomal comparative genomic hybridization

Key Words: Melanoma cell line, Fluorescence *In Situ* Hybridization, Comparative Genomic Hybridization, Spectral Karyotyping, Array CGH

Send correspondence to: Dr Margit Balazs, Department of Preventive Medicine, School of Public Health, Medical and Health Science Center, University of Debrecen, 4028 Debrecen, Kassai u 26, Hungary. Tel/Fax: 36-52-417-267, E-mail: margo@jaguar.dote.hu

http://www.bioscience.org/current/vol11.htm