Conservation of Genetic Alterations in Recurrent Melanoma Supports the Melanoma Stem Cell Hypothesis

Marianna Sabatino, Yingdong Zhao, Sonia Voiculescu, Alessandro Monaco, Paul Robbins, Laszlo Karai, Brian J. Nickoloff, Michele Maio, Silvia Selleri, Francesco M. Marincola, and Ena Wang

1Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, Biometrics Research Branch, Surgery Branch, and Pathology Department, National Cancer Institute, NIH, Bethesda, Maryland; 2Skin Disease Research Program, Loyola University, Chicago Medical Center, Maywood, Illinois; 3Division of Medical Oncology and Immunotherapy, Department of Oncology, University Hospital of Siena, Istituto Toscana Tumori, Siena, Italy; 4Cancer Bio-Immunotherapy Unit, Department of Medical Oncology, Centro di Riferimento Oncologico, Aviano, Italy; 5Department of Human Morphology, University of Milano, Milan, Italy; and 6Department of Medical Oncology, Centro di Riferimento Oncologico, Aviano, Italy; and 7Cancer Res 2008; 68: (1). January 1, 2008 122 www.aacrjournals.org

Abstract

It is generally accepted that human cancers derive from a mutated single cell. However, the genetic steps characterizing various stages of progression remain unclear. Studying a unique case of metastatic melanoma, we observed that cell lines derived from metachronous metastases arising over a decade retained a central core of genetic stability in spite of divergent phenotypes. In the present study, we expanded our previous observations comparing these autologous cell lines of clonal derivation with allogeneic ones and correlated array comparative genomic hybridization (aCGH) with gene expression profiling to determine their relative contribution to the dynamics of disease progression. aCGH and gene expression profiling were performed on autologous cell lines and allogeneic melanoma cell lines originating from other patients. A striking correlation existed between total extent of genetic imbalances, global transcriptional patterns, and cellular phenotypes. They did not follow a strict temporal progression but stemmed independently at various time points from a central core of genetic stability best explained according to the cancer stem cell hypothesis. Although their contribution was intertwined, genomic imbalances detectable by aCGH contributed only 25% of the transcriptional traits determining autologous tumor distinctiveness. Our study provides important insights about the dynamics of cancer progression and supports the development of targeted anticancer therapies aimed against stable genetic factors that are maintained throughout the end stage of disease. [Cancer Res 2008;68(1):122–31]

Introduction

It is generally recognized that cancers originate from the clonal expansion of a single mutated founder cell (1). The veracity of this statement is critical because clonal derivation may affect the effectiveness of cancer therapies targeting genetic elements passed on to each generation by parental cells throughout the natural history of the disease. Experimental animal models (2) and clinical observations support this model (3); in melanoma, clonal derivation was documented in the transition from radial to vertical growth (4), in in-transit metastases (5), and in the progression from primary to metastatic disease (6). We recently characterized melanoma cell lines derived from metachronous melanoma metastases in a patient who experienced several recurrences over more than a decade (7). Mutational analysis of β-catenin gene, methylation of the human androgen receptor, karyotyping, and chromosomal comparative genomic hybridization (cCGH) documented a clonal origin of each cell line from a single ancestor. Although all cell lines originated from the same founder, each did not likely derive from the most recent previous metastasis because aberrations in each recurrence were sufficiently different to exclude a purely sequential process. Because subsequent recurrences did not build on the genomic alterations found in the earlier one, it was reasonable to postulate that there must have been a progenitor cell more genetically stable than the cells expanded from individual metastases, supporting the melanoma stem cell postulate (8–10). Moreover, a β-catenin mutation identified in all cell lines suggested that a stable mutation responsible for heightened self-renewal properties through activation of stem cell signaling pathways (11, 12) was carried by all the descendent cells. Interestingly, cells karyotyped from the same culture revealed differences evocative of ongoing chromosomal instability within the cell population isolated from each metastasis (10). It was nevertheless of interest to note that, in spite of the genetic entropy displayed by cells cultured from subsequent metastases, a conserved core of genetic alterations was maintained throughout the natural history of the disease that could have been inherited from the progenitor stem cell or resulted from a process of convergent evolution dictated by the structural organization of the genome. Indeed, shared chromosomal imbalances have been described in melanomas derived from different patients (4, 13–15). In the previous study, we applied cCGH that cannot detect single copy gains or losses involving regions smaller than 5 to 10 Mb. For the detection of smaller genomic imbalances, in the present study, we applied array CGH (aCGH) using a cDNA platform that provides direct evaluation of coding regions and allows parallel comparisons between genomic imbalances and transcriptional patterns (16–20). By using the same microarray platform for both aCGH and cDNA expression studies, Pollack et al. (17) found that changes in gene copy number were consistently associated with corresponding changes in expression of the affected genes. Similar observations were made by Nigro et al. (19) in glioblastoma samples. Most observations were performed at a single time point relevant to either primary or metastatic lesions, although no longitudinal long-term studies have been reported due to the rarity in which...
long-term recurrences occur in cancer. Thus, we assessed the weight that genetic imbalances bore on transcriptional programs during a period spanning more than a decade. Admittedly, by studying cell lines, information derived from the whole tumor may be missed. Further attempts to rescue DNA from fixed material from which the cell lines originated failed to yield material of sufficient quality for validation of the in vivo findings, limiting the breadth of our work to an indirect analysis. However, karyotypic and genetic profile of cell lines are representative of persistent genetic alterations in vivo, as converging phenotypes are unlikely to randomly occur in vitro under steady-state culture conditions (14, 21–25).

Materials and Methods

Peripheral blood mononuclear cell preparation. Peripheral blood mononuclear cells (PBMC) were used as a reference for both aCGH and transcriptional analysis. For aCGH, PBMCs from a female donor who received leukapheresis at the Department of Transfusion Medicine were Ficoll gradient separated and used throughout the study. Validation of aCGH accuracy was done by obtaining six additional PBMCs from female and six PBMCs from male donors to confirm stability of gene representation using RNA Mini or Midi kit (Qiagen) and amplified into antisense RNA as previously described (27, 32, 33). After amplification, the quality of antisense RNA was tested with Agilent Bioanalyzer 2000 (Agilent Technologies). Total RNA was extracted from PBMCs or cell lines using RNA Mini or Midi kit. DNA labeling was conducted using BioPrime aCGH Genomic Labeling kit (Invitrogen; ref. 31). Test and reference DNA were, respectively, labeled with Cy5 (red) and Cy3 (green) and cohybridized to the cDNA Labeling kit (Invitrogen; ref. 31). Test and reference DNA were, respectively, labeled with Cy5 (red) and Cy3 (green) and cohybridized to the cDNA microarrays printed at the Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, NIH with a Ficoll gradient separated and used throughout the study. Validation of aCGH accuracy was done by obtaining six additional PBMCs from female and six PBMCs from male donors to confirm stability of gene representation using RNA Mini or Midi kit (Qiagen) and amplified into antisense RNA as previously described (27, 32, 33). After amplification, the quality of antisense RNA was tested with Agilent Bioanalyzer 2000 (Agilent Technologies). Total RNA was extracted from PBMCs or cell lines using RNA Mini or Midi kit. DNA labeling was conducted using BioPrime aCGH Genomic Labeling kit (Invitrogen; ref. 31). Test and reference DNA were, respectively, labeled with Cy5 (red) and Cy3 (green) and cohybridized to the cDNA microarrays printed at the Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, NIH with a configuration of 32 × 24 × 23 and contained 17,500 elements (see Supplementary Data). The entire aCGH data set will be available online.

Expression analysis. Total RNA was extracted from PBMCs or cell lines using RNA Mini or Midi kit (Qiagen) and amplified into antisense RNA as previously described (27, 32, 33). After amplification, the quality of antisense RNA was tested with Agilent Bioanalyzer 2000 (Agilent Technologies). Total RNA from PBMC pooled from six normal donors was extracted, amplified, and used as a reference for transcriptional studies (26, 27).

Cell line origin and culture. Five melanoma cell lines were generated from distinct cutaneous melanoma metastases that progressively appeared in patient 888 during a period spanning longer than a decade (7). The metastases were excised for diagnostic or therapeutic purposes at the Surgery Branch, National Cancer Institute (Bethesda, MD). Other melanoma cell lines were generated from metastases from other patients (28). A375 cell line was purchased from the American Type Culture Collection. Cell lines were maintained in steady-state culture conditions using RPMI 1640 (Biosource International) supplemented with 10% fetal bovine serum (Biosource International). Cell lines were harvested by trypsin/versene (Biosource International) digestion (28). High-resolution HLA typing was performed by sequence-based typing (29, 30).

Array comparative genomic hybridization. Genomic DNA was extracted from short-term cultured tumor cells or PBMCs using Qiagen Mini kit. DNA labeling was conducted using BioPrime aCGH Genomic Labeling kit (Invitrogen; ref. 31). Test and reference DNA were, respectively, labeled with Cy5 (red) and Cy3 (green) and cohybridized to the cDNA microarrays printed at the Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, NIH with a configuration of 32 × 24 × 23 and contained 17,500 elements (see Supplementary Data). The entire aCGH data set will be available online.

Data accrual and statistical analysis. Fluorescence intensities generated by Cy5 and Cy3 probes hybridized onto the microarray slides were scanned at 10-μm resolution on a GenePix 4000 scanner (Axon Instruments) at variable photomultiplier tube voltage to obtain maximal signal intensities with <1% probe saturation. The fluorescence intensity and ratio data for Cy5 and Cy3 were initially analyzed using GenePix 4.3 software. Data on log base 2 scale was normalized with Lowess smoother method using BRBArrayTools developed by the Biometric Research Branch, National Cancer Institute. Further data preprocessing was done using web tool prep. Duplicate data points were merged by taking the average over the UniGene cluster IDs. Missing data were imputed using K-nearest neighbor imputation method with K = 15. Gene location was extracted according to UniGene cluster IDs from Ensemble and University of California at Santa Cruz using IDconverter. The preprocessed aCGH data were then segmented using the circular binary segmentation method implemented in ADAhCG to detect regions with abnormal DNA copy number. The smooth variables were set as defaults. The significance level for the test to accept change points was set to be 0.01 under 10,000 permutations. The cutoff for gain/loss calls was ±0.12 on the log base 2 scale, which is consistent with others’ reports (4, 6, 34–36). Transcriptional data on melanoma cell lines were process by averaging results in replicate experiments for the same genes and aligned with aCGH data. Whole profile comparisons between aCGH and transcriptional data were performed using Spearman rank correlation. Gene annotations were extracted using IDconverter. Hierarchical clustering analysis was performed on BRBArrayTools using centered correlation as similarity metric and centroid linkage method: Cluster 3d. Heat maps were generated using Eisen’s TreeView software. Preferential chromosomal location of genes differentially expressed by patient 888 compared with allogeneic cell lines at different levels of significance (unpaired t test) was evaluated by Fisher's exact test (37) or by applying the BRBArrayTools gene set analysis in which the statistical significance (P value) of a gene set containing a given number (n) of genes is evaluated using the likelihood score (Kolmogorov-Smirnov) statistic permutation test for gene enrichment analysis (38). The test was separately applied to each gene set differentially expressed between patient 888 and the allogeneic cell lines within each chromosome or in areas of imbalances as detailed in Results.

Results

Patient History

Details about the patient history were previously reported (7). Briefly, patient 888 had a congenital mole removed in December 1988. Over a period of more than a decade, the patient experienced several recurrences (Supplementary Table S1); from each one a melanoma cell line was established. Because we previously documented a clonal origin of the cell lines (7), we sought the opportunity to study the dynamics of genetic variation and its relationship with transcriptional changes in this case with such a uniquely prolonged course.

Genomic Imbalances

Definition of chromosomal gains and losses. The threshold for chromosomal region gains and losses was determined comparing female with male PBMC (Fig. 1A–C). Six sex-matched female and six sex-mismatched male genomic DNAs were individually cohybridized against one normal female genomic DNA that served as a constant reference. A ratio of ≥0.12 was determined as the threshold for at least one gene copy gain (+) or loss (−) corresponding to an exclusion of the 99th percentile of autosomal data while eliminating chromosome X ratios under the 15th percentile. Differences in gene copy number for chromosome Y could not be statistically assessed due to the small number of loci from this chromosome spotted in the array chip not allowing for sufficient statistical power.

Specificity of chromosomal imbalances in patient 888 melanoma cell lines. The previously defined threshold was used to identify chromosomal imbalances in cell lines derived from patient 888 and other patients (28, 39, 40). The imbalances observed in patient 888 cell lines are portrayed graphically in Fig. 1D and summarized in Table 1.

All melanoma cell lines from patient 888 bore genomic imbalances in one or more DNA regions; some of them were similar supporting their common derivation from a unique ancestor. High concordance was observed between the previous data obtained by cCGH (7) and the present analysis based on aCGH (Fig. 2A and B). A primary cluster of imbalances, including −10p, −17p, +3p, −7, and +17q, was confirmed by this study; linkage clustering applied to cCGH data had originally attributed this cluster to the hypothetical founder stem cell (7). Small deletions in chromosome 9p, 14q, and 16q were consistently seen in patient 888 cell lines by aCGH that had been less consistently seen by cCGH analysis (7). Moreover, unsupervised hierarchical clustering of patient 888 and allogeneic cell lines based on aCGH clearly segregated the former from the latter, strongly supporting patient specificity independent of disease stage (Fig. 2B). However, within patient 888 cluster, a separation was noted between 888-MEL and subsequent cell lines, suggesting that the biggest evolutionary step in the dedifferentiation process occurred early on. Although the global genetic make up of patient 888 cell lines was highly specific, commonalities were observed with allogeneic cell lines best explained by a convergent tendency of some imbalances to occur at higher rates in specific chromosomal regions as a reflection of a general pattern of chromosomal instability of human melanoma. Losses in chromosome 9, 10, and 17 and gains in chromosome 7 were respectively seen in three, five, five, and six of eight allogeneic cell lines. This suggests that these imbalances may bear particular significance in the genesis of melanoma as also observed studying NCI-60 cell lines (14). Interestingly, most of the common alterations

![Figure 1. Graphic illustration of aCGH smoothed data from normal donor and melanoma cell lines. A and B, representative examples of aCGH based on genomic DNA from female (A) and male (B) healthy donors cohybridized to a cDNA array platform together with a genomic DNA from a female donor used as constant reference in all experiments performed in this study. Log(2) ratios were plotted on the Y axis for all clones on the array according to their map position (X axis). A ratio of ±0.12 was determined as the threshold for at least one gene copy gain (+) or loss (−) corresponding to an exclusion of the 99th percentile of autosomal data while eliminating chromosome X ratios under the 15th percentile. The colors used for each target sequence are yellow (no significant DNA copy number change) and green (significant DNA loss). C, the complete aCGH data for the 22 autosome and the X chromosome averaging a series of six female-female and six female-male hybridizations using genomic DNA obtained respectively from six female and six male normal donor PBMCs. D, whole-genome aCGH profile for patient 888 melanoma cell lines. Log(2) ratios were plotted on the Y axis for all clones on the array according to their map position (X axis). A log(2) ratio threshold of 0.12 was used to determine clones gained (+0.12) or lost (−0.12). The colors used for each target sequence-specific dot outline areas of no significant DNA copy number change (yellow), significant DNA loss (green), and significant DNA gain (red).](#)
Table 1. Genomic loss/gain in melanoma cell lines as obtained from aCGH analysis

<table>
<thead>
<tr>
<th>Chromosome location</th>
<th>888</th>
<th>1290</th>
<th>1858</th>
<th>1936</th>
<th>1962</th>
<th>A375</th>
<th>526</th>
<th>1102</th>
<th>1182</th>
<th>1195</th>
<th>938</th>
<th>624-28</th>
<th>624-38</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** ↑ and ↓ indicate gains or losses, respectively; chromosomal coordinates are in subscript text with p and q denotation only when the imbalance spans across the centromere. Larger font and color codes on the side identify imbalances common (at three of five cell lines) in patient 888 (red, gains; green, losses). → refers to imbalances considered to be most representative of patient 888's disease. Green highlights at the head of each row denote deletions common in patient 888 cell lines (at least three of five cell lines). Red highlights at the head of each row denote gains common in patient 888 cell lines (at least three of five cell lines).

*Imbalances previously identified by chromosomal CGH in patient 888.

identified by our study seem to be peculiar for melanoma as they were not frequently seen among the NCI-60 cell lines of other histology (14).

Other chromosomal aberrations observed frequently in the NCI-60 melanoma cell lines (14) were not seen in patient 888; these included losses in chromosomes 4, 11, and 18 and gains in chromosome 20, which were seen in five, three, and six of eight allogeneic cell lines. Finally, other chromosomal aberrations described in the NCI-60 melanoma cell lines were not confirmed by this study, including losses in chromosomes 13, 14, 16, and 21 and gains in chromosomes 2 and 3 (14). In summary, although patient 888 cell lines shared genetic traits with other allogeneic cell lines and with historical comparisons with the NCI-60 melanoma cell lines, their global profile portrayed a distinctive fingerprint that persisted throughout the natural history of the disease.

Dynamics of chromosomal alterations in patient 888. Significant losses in chromosomes 1p, 8p, 9p, 10p, 14q, 16q, 17p, and 22q and gains in chromosomes 3p, 7q, 8q, and 17q were detected in most of patient 888 cell lines (Table 1). Expectedly, the number of genomic regions with statistically determined genetic imbalances increased with time going from a total of 11 in 888-MEL to 23 in 1936-MEL. However, this trend was not consistently
maintained because the last cell line 1962-MEL contained only 14 imbalances. Interestingly, this observation paralleled the strongly dedifferentiated phenotype of 1936-MEL, which displayed, among sister cell lines, the most degenerate aCGH profile (Fig. 2B), strong transcriptional deviation (Fig. 2C and D), total loss of pigmentation (Fig. 3B), and an atypical non–spindle-type morphology in culture (Fig. 3C).

The length of individual genomic imbalances also increased with time. Deletions of chromosome 9p well exemplify the dynamics of the extension of genomic alterations (see Supplementary Fig. S4). Losses of 9p progressively extended throughout the natural evolution of the disease from a limited region spanning 9p21-22, which surrounds the CDKN2A melanoma susceptibility locus (41, 42). This extension, however, did not occur in a strictly sequential fashion, once again emphasizing the relatively independent behavior of individual metastasis progression from the central ancestor. Interestingly, a two-sample t test (\( P < 0.01 \)) applied to aCGH data to partition cell lines derived from early-stage tumors that responded to immunotherapy (888-MEL and 1290-MEL) from the later unresponsive ones (1858-MEL, 1936-MEL, and 1962-MEL) identified the genomic region around 9p21 as most representative of this watershed stage of the disease. This may be of interest because this area encodes several genes related to melanoma differentiation, immune responsiveness, and prognosis (41–43).

Most genetic alterations followed a similar pattern of individual extension around a common center core (Table 1). The sporadic and nonsequential acquisition of additional genetic imbalances is exemplified by gains in chromosome 20q that were observed in 888-MEL, 1936-MEL, and 1962-MEL and in five of eight allogeneic cell lines but were missing in the intermediate sister cell lines.

**Transcriptional Profiling**

Each cell line used in this study was thawed, cultured, harvested, and processed for mRNA isolation, purification, and hybridization to the cDNA array chips twice. Transcriptional analysis was applied by averaging data from the two independent experiments to account for variability in mRNA expression during in vitro culture. Unsupervised clustering based on the complete data set applying
center/centroid linkage analysis identified two main clusters: one containing all the cell lines originated from patient 888 and the other including only allogeneic ones (Fig. 2C). The former included also two allogeneic cell lines (938-MEL and the two 624-MEL clones) that clustered at a relatively close Euclidian distance from patient 888 cell lines. Among patient 888 cell lines, those derived from the same lesion clustered together. 888-MEL clustered closer to 1858-MEL, whereas 1290-MEL clustered at a higher Euclidian distance. Three different cell lines expanded following different procedures from the same 1858 metastasis clustered together. Similarly, two different passages of the 1290-MEL cell line (an early and a later passage) clustered together excluding the occurrence of clonal selection during prolonged cell culture that might have affected their genetic profile (25). The later cell lines 1936-MEL and 1962-MEL that grouped progressively away from the primary cluster still remained transcriptionally closer to the sister cell lines than the allogeneic ones. Strong similarity was observed between the global transcriptional differences observed by unsupervised clustering and the level of differentiation of each cell line based on the transcriptional expression of melanoma differentiation antigens (Fig. 3A); 888-MEL and 1858-MEL displayed the highest level of expression compared with 1290-MEL and the subsequent cell lines (44). As previously mentioned, 1936-MEL displayed a phenotype in culture quite different from its sister cell lines characterized by lack of pigmentation and a rounded morphology in vitro compared with the spindle melanocyte-resembling morphology of the others. The anaplastic behavior of this cell line had also been associated in our previous study (7) with an unusual level of chromosomal instability, high level of triploid/tetraploid chromosomal counts. A tight correlation between transcriptional and protein expression was observed comparing the present data with our previous analysis of melanoma antigen expression in the same cell lines by intracellular fluorescence-activated cell sorting analysis. With the exception of 526-MEL, the expression of the Melan-A and gp100/PMel17 (silv) correlated closely with previously described protein levels (28).

**Correlation between Genomic Imbalances and Gene Expression Profiling**

Cumulative comparison by Spearman rank correlation of aCGH with transcriptional data showed in several cases a significant or close to significant correlation (Table 2).
888 cell lines with increased average expression of the respective, losses of chromosome 9 were correlated in patient with amplifications and decreased with losses. However, few number of genes to allow bigger statistical power. Rest of the genes included in the arrays. In several cases, there was a ratios of the genes belonging to the region were compared with the

Cancer Research

performing a two-sample t test in which the average intensity

Table 2. Cumulative comparison of aCGH with transcriptional data

<table>
<thead>
<tr>
<th>Chromosomal location</th>
<th>Pearson 888</th>
<th>1290p14</th>
<th>1290p54</th>
<th>1858</th>
<th>1858</th>
<th>1858</th>
<th>1936</th>
<th>1962</th>
<th>A375</th>
<th>526</th>
<th>1102</th>
<th>1182</th>
<th>1195</th>
<th>938</th>
<th>624-28</th>
<th>624-38</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1q21.1 to p34.1</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2q37.3 to q37.3</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3p26.3 to p21.31</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3q26.2 to q26.2</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4p15.1 to q35.2</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5p15.33 to p12</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5q11.2 to q34</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6p25.3 to p21.33</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6q16.3 to q21</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7p22.3 to q11.23</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7q11.23 to q36.3</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8p23.3 to q24.3</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8q24.3</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9p23 to q12</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10p14 to q23</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11p12 to p15</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11p11.2 to q13.2</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11q14 to q23.3</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12p11.2 to p13.31</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12q22 to q24.13</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13q13.3 to 13q34</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14q22.2 to q32.33</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15q13.3 to q26.3</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16q12.1 to q22.1</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16q22.1 to q23</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17p13.3 to p12</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17p12 to q21.2</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18p11 to q11.2</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18q11.2 to q23</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20p13 to q13.33</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20q11 to q13.33</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: | and | indicate gains or losses, respectively; chromosomal coordinates are in subscript text with p and q denotation only when the imbalance spans across the centromere. +, direct correlation; —, inverse correlation; + or —, P < 0.05; ++ or ——, P < 0.01; +++ or ———, P < 0.001. — refers to imbalances considered to be most representative of patient 888's disease. Green highlights at the head of each row denote deletions common in patient 888 cell lines (at least three of five cell lines). Red highlights at the head of each row denote gains common in patient 888 cell lines (at least three of five cell lines).

Analysis of individual segments was performed by identifying genes within each aCGH-defined region of imbalance and performing a two-sample t test in which the average intensity ratios of the genes belonging to the region were compared with the rest of the genes included in the arrays. In several cases, there was a good correlation between individual imbalances and transcriptional data particularly when the region included a sufficiently large number of genes to allow bigger statistical power.

Most correlations were direct because transcriptional changes matched the direction of the imbalance (increased ratios associated with amplifications and decreased with losses). However, few examples of consistent inverse correlation were noted: in particular, losses of chromosome 9 were correlated in patient 888 cell lines with increased average expression of the respective genes. Although this inverse correlation reached statistical significance only in two cell lines (Table 2), the same trend was consistently observed in all patient 888 cell lines and none of the allogeneic lines. Because aCGH is insensitive to structural chromosomal changes, such as translocations, and it represents a virtual gene-by-gene reconstruction of hypothetical chromosomes, this finding cannot be explained by structural rearrangements of the genome but it rather may represent a compensatory mechanism to allelic loss.

To test whether the observed correlations between aCGH and transcription exclusively determined the segregation of the sister cell lines from the allogeneic ones, we then pooled from the complete transcriptional data set genes encoded within the genomic regions of chromosomes 7, 9, 14, and 17 containing imbalances statistically associated with gene expression; these genomic regions of chromosomes 7, 9, 14, and 17 were considered to be most representative of patient 888's disease. Green highlights at the head of each row denote deletions common in patient 888 cell lines (at least three of five cell lines). Red highlights at the head of each row denote gains common in patient 888 cell lines (at least three of five cell lines).
autologous and allogeneic cell lines. As expected, this exercise
clearly separated patient 888 from allogeneic cell lines. Surprisingly,
however, the remaining genes not included in these regions could
still segregate the sister cell lines away from allogeneic ones,
suggesting that correlations between genetic imbalances and gene
expression may participate in determining the genetic identity of
each patient’s cancer but are not the only factor.

To explain this finding, we tested whether the genes determining
patient 888 individuality were randomly distributed across the
whole genome or had preferential chromosomal locations. A two-
sample t test was applied to identify genes differentially expressed
between all of patient 888 melanoma cell lines (independent of
stage of disease) and allogeneic cell lines at several levels of
significance. Subsequently, a Fisher’s exact test was applied in a
two-by-two table to test if the number of differentially expressed
genes in each chromosome was significantly higher than that of
genes not differentially expressed (37). At a \( \chi^2 \) value of 3.841
(determined for a \( P < 0.05 \) when the degree of freedom is 1), only
chromosome 17 displayed a significant enrichment of genes
differentially expressed between patient 888 and other patients’
cell lines. Similarly, the Kolmogorov-Smirnov statistic permutation
test for gene enrichment analysis (38) showed a significance
expression bias only in chromosome 17. This finding may reflect
the large size of genomic imbalances affecting this compared with
other chromosomes.

We then measured the relative contribution of genes contained
in areas of genetic imbalance detectable by aCGH to the global
transcriptional identity of autologous cell lines. For this purpose,
regions with consistent chromosomal imbalances across the
patient’s history were defined those statistically significant in at
least three of the patient cell lines (Table 1). The genes included in
all these regions were pooled and tested against the rest of the gene
expression data set. We calculated for each of the two so-defined
groups the number of genes differentially expressed between
patient 888 and the allogeneic cell lines at different level of
significance (<0.01, <0.05, unpaired two-tailed t test).
At all stringency levels, regions of imbalance were enriched with
differentially expressed genes compared with the rest of the
genome \((\chi^2 \) value of 22.5, 20.5, and 11.7 for the three levels of t test
significance for a \( \chi^2 \) < 0.001 in all cases; Table 3A). At decreasing
stringency, the difference in the proportion of genes differentially
expressed between patient 888 cell lines and allogeneic cell lines
in the two regions decreased (4% versus 2% at a t test \( P < 0.001,
12\% versus 9\% at \( P < 0.01), \text{ and } 25\% \text{ versus } 21\% \text{ at } P < 0.05). \text{ Similar}
results were obtained with the BRBArrayTools gene enrichment
module (\( P < 0.001\); Table 3B). Importantly, although the regions of
imbalance were enriched with differentially expressed genes, they
accounted only for a small proportion of the transcriptional
signatures determining patient 888 cell line identity. In all cases,
the total number of differentially expressed genes was lower
compared with regions not containing imbalances and their
contribution was limited to 26.8\%, 20.9\%, and 17.9\% at
the respective levels of significance. Thus, our study suggests that
expression biases due to genetic imbalances contribute about 20%
to 25% of the genetic fingerprint of individual patients’ tumors and
this balance persists throughout the end stage of disease.

Discussion

The natural history of cancer is likely determined by the
accumulation in time of genetic alterations within a cell lineage
(45). However, the recently proposed cancer stem cell hypothesis
fine tuned this belief, suggesting that such alterations do not follow
a linear progression from one tumor to the next but rather, like
branches from a tree, stem independently from a common
progenitor cell. This progenitor stem cell is responsible for
preserving the genetic traits necessary for long-term survival,
whereas its progeny follows an independent pattern of dedifferen-
tiation in adaptation to tissue-specific requirements.

Chromosomal aberrations have been described in human
malignant melanoma using conventional cytogenetic methods (4)
and associated with candidate predisposition loci (13, 15, 41, 42,
46–49). Most of the information is representative of a single time
point, whereas little is known about the evolution of genetic
alterations in time and how this may affect the evolving phenotype
of cancer cells. This is due to the rarity in which long-term analysis
of recurrent disease can be performed in this rapidly killing cancer.
The model used here allowed the assessment of the evolution of
metastatic melanoma throughout a decade of recurrences (7),
confirming that a direct correlation exists between genetic
background and transcriptional profile. This correlation is specific
to each imbalance and does not change with time. However, such
correlation could not be consistently predicted for all imbalances
and, on occasion, inverse correlations could be observed for which
a good explanation is lacking. Most importantly, transcriptional
patterns related to genetic imbalances detectable by aCGH
accounted only for a limited proportion (20-25\%) of the functional
signatures that determined the individuality of this patient’s cancer
natural history.

This study suggests that, in spite of a certain degree of entropic
degeneracy restricted to each cell line, a strong core of genetic
stability dominated the genetic make up of this tumor along the
12-year period that caracterized her metastatic history. This
stability insensitive to budding variations supports the cancer
stem cell hypothesis (10). In this patient’s case, the disease likely
started with a relatively well-differentiated tumor expressing high
levels of melanoma differentiation antigens, a melanocyte-like
phenotype in culture, and limited genetic alterations (888-MEL).
Whether this characteristic led the tumor immune responsiveness
to treatment with a tumor-infiltrating lymphocyte (TIL) recogniz-
ing one of these antigens (tyrosinase) is impossible to test in a
single case study. The second lesion, 1290-MEL, remarkably lost
the expression of most tumor differentiation antigens, the
melanocytic appearance in in vitro culture, and was genetically
and transcriptionally less differentiated than 888-MEL and the
subsequent cell line. Yet, it maintained the ability to respond to
treatment with a TIL population reactive against a mutated
protein not associated with the melanocytic lineage but likely
essential for cancer cell survival (\( \beta \)-catenin; ref. 11). 1858-MEL was
genetically and phenotypically closest to 888-MEL, although
deriving from a metastasis that appeared a decade later and did
not respond to immunotherapy. It is likely that the phenotype
of these cell lines is representative of in vivo conditions as three
independent cell lines prepared and maintained close proximity to
each other after long-term culture. In spite of this high degree of
differentiation, 1858-MEL completely lost surface expression of
HLA class I molecules because of a genomic loss of the 15q21.1-
22.1 region where the \[ \beta_2 \text{-microglobulin} \] locus is situated.15 This

---

15 Manuscript in preparation.
represents an emblematic example of a molecularly defined escape mechanism that may play a dominant role in determining responsiveness to treatment (50, 51).

In spite of this phenotypic diaspora, a central core of genetic alterations was maintained through the end of the patient’s life (Fig. 2A). It is possible that within this conserved core of genetic stability rests the interpretation of this patient’s fate and the Achilles’ heel of her cancer. Indeed, we believe that this in vitro documentation is representative of the evolution of this patient tumor. It is unlikely that the consistent alterations documented here were due to converging genetic artifacts related to in vitro culture because patient 888 cell lines maintained their individuality compared with allogeneic lines also grown in vitro for a long time.

In spite of the limited effect (25%) that genetic imbalances have on the transcriptional pattern of this patient’s cancer, their global patterns correlated well with mRNA patterns and phenotypes; cell lines displaying enhanced level of genetic degeneracy (such as 1936-MEL) displayed also most severe transcriptional, translational, and phenotypic changes. This suggests that downstream cellular functions that cannot be directly accounted for by chromosomal alterations may be indirectly related through modulation of regulatory mechanisms, such as methylation patterns, mutational analysis, and characterization of microRNA.

In summary, the study of chromosomal imbalances based on aCGH confirmed our previous observations (7). Moreover, this study suggests that (a) both DNA- and mRNA-determined patterns influenced the individuality of this patients’ tumors; (b) the individuality was maintained through the end stages of disease; (c) although their contribution is intertwined, DNA and mRNA are independent factors with genomic imbalances detectable by aCGH, contributing only 25% of the transcriptional traits determining autologous tumors singularity; (d) yet a striking correlation existed between total extent of genetic imbalances, global transcriptional

---

**Table 3. Expected over observed enrichment of genes determining patient 888 identity in regions of genomic imbalances**

<table>
<thead>
<tr>
<th>A. ( \chi^2 ) test</th>
<th>No. genes</th>
<th>% Differentially expressed over total</th>
<th>% Differentially expressed in regions of imbalance over total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Differentially expressed</td>
<td>Not differentially expressed</td>
<td>Total</td>
</tr>
<tr>
<td>Imbalance regions</td>
<td>61</td>
<td>1,313</td>
<td>1,374</td>
</tr>
<tr>
<td>Rest of genome</td>
<td>167</td>
<td>7,404</td>
<td>7,571</td>
</tr>
<tr>
<td>Whole genome</td>
<td>228</td>
<td>8,717</td>
<td></td>
</tr>
<tr>
<td>( \chi^2 = 22.47 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( \Delta/\text{total} )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Kolmogorov-Smirnov statistic permutation test</th>
<th>No. classes: 2</th>
<th>No. genes that passed filtering criteria: 9,881</th>
<th>Type of univariate test used: two-sample ( t ) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorted by ( P ) value of the likelihood score permutation test</td>
<td>The first category (genes in imbalances) was significant at the nominal 0.001 level of the likelihood score permutation test or Kolmogorov-Smirnov permutation test</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>No. genes</th>
<th>Gene list</th>
<th>Likelihood score permutation ( P ) value</th>
<th>Kolmogorov-Smirnov permutation ( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,518</td>
<td>C:/Program Files/ArrayTools/Genelists/User/Imbalance/ImBalancedSet.txt</td>
<td>( P &lt; 1e-07 )</td>
<td>( 2.00e-04 )</td>
</tr>
</tbody>
</table>
patterns, and cellular phenotypes; and (e) genetic imbalances, transcriptional patterns, and phenotypes did not follow a strict temporal progression but stem independently at various time points from a central core of genetic stability best explained according to the cancer stem cell hypothesis. Thus, the present study may provide important insights about the dynamics of cancer progression and support the development of targeted anticancer therapies aimed at stable genetic factors maintained through the end stage that determine the individuality of each patient’s disease.

Acknowledgments
Received 5/24/2007; revised 8/16/2007; accepted 10/6/2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References
18. Furge KA, Dykema KJ, Ho C, Chen X. Comparison of array-based comparative genomic hybridization with gene expression-based regional expression biases to identify genetic abnormalities in hepatocellular carcino-
Correction: Melanoma Stem Cell Hypothesis

In the article on melanoma stem cell hypothesis in the January 1, 2008 issue of Cancer Research (1), the affiliations for Dr. Alessandro Monaco should be listed as Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, NIH, Bethesda, Maryland and Clinical Experimental Oncology Laboratory, National Cancer Institute Giovanni Paolo II, Bari, Italy.

Conservation of Genetic Alterations in Recurrent Melanoma Supports the Melanoma Stem Cell Hypothesis

Marianna Sabatino, Yingdong Zhao, Sonia Voiculescu, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/1/122

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2007/12/27/68.1.122.DC1

Cited articles
This article cites 51 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/1/122.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/68/1/122.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.