Nonrandom Chromosome Changes in Malignant Melanoma

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ABSTRACT

Chromosome aberrations were analyzed in 4 cases of malignant melanoma (MM) after disaggregation of the tumors with collagenase and short-term culture. In all cell cultures, the MM cells displayed a typical triangular spindle form.

The chromosome number was near-diploid in one case and near-triploid in three cases. A total of 27 abnormal chromosomes were identified with the Giemsa banding technique. By far, the most common types of abnormalities were translocations, followed by deletions and isochromosomes. Chromosomes 1, 6, and 7 were found to be most frequently involved in structural aberrations. Markers originating from chromosomes 1 and 6 were found in all four cases, and abnormalities of chromosome 7 were found in three. Each marker chromosome was unique for a given case; no common markers for two or more cases were found.

Based on the present results and an analysis of reports on the chromosomal constitution of MM cells in the literature, we suggest that abnormalities involving chromosomes 6 and 7 may be a characteristic feature of MM. Aberrations of chromosome 1, although common in MM, may be part of a general cytogenetic feature in human neoplasia.

INTRODUCTION

Difficulty in obtaining a sufficient number of metaphases (quantitative) and the poor quality of most metaphases hampered detailed chromosome analysis of solid tumors in the past. Major progress has recently been made in this area through the introduction of enzymatic disaggregation of tumor tissue for cytogenetic studies. The use of collagenase and trypsin reported earlier by this laboratory (20) considerably improved the yield of viable tumor cells, as compared to conventional mechanical disaggregation methods. Another important improvement in the cytogenetics of malignant cells was provided by a new G-banding procedure utilizing Wright’s stain (22), which yields a higher resolution of banded chromosomes and, thus, a more exact identification of chromosomal abnormalities, thereby reducing the number of so-called unidentified markers.

Utilizing these newer techniques, it has been possible to gather more detailed cytogenetic data on solid tumors, underlined by recent findings of nonrandom aberrations in Wilms’ tumor (6), serous cystic ovarian cancer (19), lung cancer (21), and large-bowel cancer (2). MM has been the subject of a number of cytogenetic investigations in the past (1, 3, 5, 12, 15, 16, 18). Despite the identification of numerous abnormalities, no aberrations specifically connected with MM were established. We report here a detailed cytogenetic analysis of 4 MMs, the data of which suggest that chromosomes 6 and 7 are nonrandomly involved in karyotypic aberrations in MM.

MATERIALS AND METHODS

Patients

Case 1. The patient is a 54-year-old black male who was admitted to RPMI because of small bowel obstruction. He underwent surgery which revealed extensive lesions of metastatic melanoma. Multiple lesions were removed. The primary lesion remains unknown. Postoperatively, the patient received cytosstatic treatment with dacarbazine and cis-platinum. Because of progressive disease in the right lower lung, a thoracotomy became necessary, and part of the tumor removed was used for chromosome analysis.

Case 2. The patient, a 44-year-old white male, had a mole on his right leg for many years which started bleeding in May 1980. A biopsy revealed MM and, consequently, wide-local excision and superficial groin dissection were performed. One year later, multiple cutaneous nodules appeared. Immunotherapy with Bacillus Calmette-Guérin was unsuccessful. He first presented at RPMI with a metastasis to the right leg, which was resected, part of which was submitted for chromosome analysis. The patient had not received chemotherapy or radiation treatment prior to the initial cytogenetic studies.

Case 3. A metastatic lesion in the right axilla was removed from this 72-year-old female patient; pathological examination revealed a MM. Part of this lesion was submitted for cytogenetic analysis. No evidence of a primary lesion was found; however, the patient did note a pigmented skin lesion in the area of the present metastatic lesion approximately 3 or 4 years ago. The patient had not received any treatment or radiation before the axillary lymphadenectomy.

Case 4. The patient, a 46-year-old white male, was operated on for 2 primary lesions of MM on his right arm (Clark Level III; thickness, 1.15 mm) and shoulder (Clark Level I) 5 years ago, when he underwent prophylactic axillary lymph node dissection, which was negative. Recently, he developed a nodule in his left lower lung and had a wedge resection, revealing additional multiple seeds in the pleura. Because of further progression and new metastatic lesions, chemotherapy with cis-platinum and dacarbazine was started (3 courses were administered). When intraabdominal bleeding occurred, caused by extensive invasive tumor growth, an emergency laparotomy became necessary. Part of the resected tumor material was provided for chromosome analysis.

Disaggregation

The sterile specimens were immediately transferred to the cytogenetic laboratory and kept in RPMI Medium 1640 containing 20% fetal calf serum and gentamicin (Sigma Chemical Co., St. Louis, Mo.; 50 μg/ml). The disaggregation of tumors was accomplished by a modified version of a method reported previously (20). The specimens were first mechanically minced with scissors and scalpels into small pieces approximately 2 to 3 mm in diameter and incubated in collagenase (Worthington Biochemical Corp., Freehold, N. J.; stock solution consisted of 80 mg of collagenase II in 10 ml of balanced salt solution) at 37°. After 1.5 hr, the cell suspension was gently passed through a 100 mesh screen, with light pressure being applied by a finger coated with a sterile surgical glove, and washed twice in RPMI Medium 1640.
Culture and Harvest

Roughly $10 \times 10^6$ cells were distributed in each of the disposable culture flasks and cultured in RPMI Medium 1640 containing gentamicin (50 $\mu$g/ml) and 20% fetal calf serum. The cells were harvested after 2 and/or 3 days, preceded by Colcemid treatment (0.02 $\mu$g/ml) overnight for 16 to 20 hr. Cells were removed from culture flasks with a rubber policeman and exposed to hypotonic treatment (0.75 M KCl) for 30 min and then fixed in methanol:acetic acid (3:1), washed twice, and stored in a freezer before the preparation of slides.

G-Banding

Slides were prepared after a minimum of 1 day of storage of the fixed material in the freezer. Three drops of a thin cell suspension were dropped from a 50-cm distance onto dry, precleaned slides and immediately dried on a hot plate (60°C). Slides were then incubated in an oven for 24 hr at 60°C.

G-banding was obtained with Wright’s stain diluted in phosphate buffer (1:3) pH 6.8, without any pretreatment, as described by Yunis (22). The proper staining time ranged from 2 to 4.5 min, depending on the age of the slides.

RESULTS

Chromosome Analysis

Case 1. All 33 metaphases analyzed were abnormal. Most cells were slightly hyperdiploid with a main mode of 47 chromosomes. A total of 14 marker chromosomes were found in 8 banded karyotypes, 13 of which are shown in Fig. 1. The number in the first parentheses indicates in how many of the 8 karyotypes studied markers M1 to M14 were found.

M1(5) t(1;8)(1pter→1p13::8p12→8qter)
M2(8) del(1)(p22)
M3(8) t(1;2)(2pter→2p23::1p22→1p363)
M4(8) t(2;3)(2pter→cen→3pter)
M5(6) 4p+; t(1;4)?
M6(5) t(2;4)(2pter→2q142::4q25→4pter)
M7(5) t(6;7)(q23;?)
M8(6) t(6;7)(6pter→cen→7qpter)
M9(7) t(2;10)(2qter→2q14::10q25→10p11)
M10(7) t(12;7)(q24;?)
M11(5) idic(16)(p13)
M12(3) t(3,16)(3pter→3q262::16p13→16qter)
M13(6) t(10;17)(10pter→cen→17qter)
M14(3) t(3,17)(17qter→17q11::3p11→3q26)

Case 2. The chromosome number was established in a total of 48 metaphases, revealing a mode in the triploid range (72 chromosomes).

Four consistent markers, shown in Fig. 2, were found:

M1 t(1;7)(1qter→cen→7qter)
M2 i(6p)
M3 t(7;21)(7pter→cen→21qter)
M4 t(15;7)(q15,?)

A total of 13 G-banded karyotypes was analyzed, and each was shown to contain 2 copies of M1 and M3; however, only 1 copy of M2 was present. M4 was present in 6 karyotypes.

Three months after the first biopsy, the patient was operated on again, and the cytogenetic analysis was repeated. The second investigation yielded identical results, including marker chromosomes M1 to M4. There was no evidence of further karyotypic evolution.

Case 3. The mode was very flat in this case. Most metaphases were found to have a chromosome number in the triploid range (61 to 68 chromosomes/metaphase). Seven cells among the 39 cells counted were near-tetraploid. One of 4 karyotypes analyzed was normal.

Markers chromosomes, shown in Fig. 3, were observed:

M1 t(3;6)(3pter→3q29::6q13→6q21)
M2 t(4;7)(4qter→cen→7pter)
M3 del(6)(6qter→6q21::6q13→6pter)
M4 t(1;13)(1pter→13q21::13q34→13pter)

Markers M1, M2, and M3 were found in all 3 karyotypes analyzed, and M4 was found only in 2.

Case 4. A total of 38 metaphases was studied, and 12 G-banded karyotypes were analyzed. Most metaphases were found to be in the triploid range, with the majority of the cells containing between 65 and 68 chromosomes. Each of the 5 marker chromosomes was detected in all 12 karyotypes; M1 was always present in 2 copies, whereas M2 and M3 were single and M4, as well as M5, were found in 1 or 2 copies/karyotype.

Description of markers shown in Fig. 4:

M1 i(1q)
M2 t(5;6)(5pter→5q35::6q13→6qter)
M3 isochromosome of the long arm of marker M2
M4 del(6)(pter→q13::q23→qter)
M5 del(6)(pter→q13)

Because the constitution of markers was almost constant in this case, the number of chromosomes per metaphase varied due to the presence or absence of additional normal autosomes.

The marker chromosomes from all cases related to chromosomes 1, 6, and 7 are shown in Fig. 5.

DISCUSSION

As shown by others (15, 18) for permanent cell lines, cells of MM can readily be identified by their typical morphology. This was also found to be the case in our study after short-term culture. The cells displayed mostly spindle or triangular shapes, which seems to be a primary characteristic of MM. This characteristic spindle or triangular morphology of cells in culture was seen in each of the cases studied by us, regardless of the type of lesion, therapy background, and nature of the primary lesion.

Chromosome analyses of MM utilizing banding techniques have been reported thus far mainly on cell lines, demonstrating that the chromosome data provide a proper assay for the identification and monitoring of such cell lines. However, since additional aberrations can become apparent during long-term culture, studies based at establishing nonrandom primary chromosome aberrations in MM should preferably be performed on direct preparations, not only putting in proper perspective the karyotypic findings obtained on long-term cultures of MM cells but also supplying cytogenetic data in a much shorter period of time. The latter element could be crucial and useful if and when the chromosomal changes are utilized as an important parameter in the therapy and clinical handling of MM.

In the cases studied by us, we found frequent involvement of chromosomes 1, 6, and 7 in different structural rearrangements. The importance of changes affecting chromosome 1 in solid tumors has already been stressed previously (5, 13, 17); these seem to be a general and frequent feature in human neoplasia. Frequently, aberrations of chromosome 1 (e.g., total or partial
trisomy) result in an excess of genetic material of the 1q arm (5), possibly endowing malignant cells with a growth advantage over their chromosomally normal competitors. Another mechanism for cells to obtain growth advantages appears to be multiplication of genetic material via the formation of isochromosomes, as shown in Case 4 for 1q. An i(1q) in MM has also been described by others (5, 12). However, the frequent involvement of chromosome 1 in human neoplasia, in the form of either numerical or morphological changes, appears to lack specificity regarding disease states, the changes ranging over a wide variety of cancers and leukemias. The areas of chromosome 1 which showed the highest susceptibility to breakage with or without structural rearrangements in our cases and those of others (5, 12, 15, 18) were the centromeric region, band p36, bands p31 to p22, and bands q21 to q31.

Chromosomes 6 and 7 were also frequently involved in structural aberrations in the cases studied by us. Chromosome 6 seems to be of particular interest in this tumor, since the genes for the major histocompatibility complex, including HLA, are located on this chromosome, and MM is one of the few tumors which reportedly can be recognized by the body's own immune system, leading to a response with detectible antibody production (8, 9). In addition, a close relationship had been reported in familial MM between the occurrence of the disease and segregation of specific HLA alleles (4, 14). Surprisingly, detectable structural aberrations of chromosome 6 seldom appear to involve the p arm itself. It is, therefore, unlikely that the chromosomal aberrations of chromosome 6 could affect the expression of the main histocompatibility complex located on the short arm of this chromosome. More data on the genetic map of chromosome 6 are needed in order to clarify the possible link between aberrations of that chromosome and the changes in gene expression which could lead to the development of MM. The aberrations of 6q were found to be mostly deletions, revealed in our study to be interstitial (Cases 3 and 4) or terminal ones (Case 4). An atypical chromosome 6 was observed in Case 3 which was characterized by an unusually prominent light band q21 and an atypical pattern of the distal part of 6q.

Aberrations of chromosome 6 were reported recently as a characteristic feature of unilateral retinoblastoma (7). The most common aberration was an i(6p), found in 6 of 9 cases of sporadic unilateral retinoblastoma and, thus, resulting in an increased amount of genetic material contained in the short arm of chromosome 6. In our 4 cases, the break points on chromosome 6 occurred in the long arm in 6 of 8 different aberrations. We observed an isochromosome of 6p in only 1 case.

Chromosome 7 was also nonrandomly and frequently involved in structural aberrations. The most frequent breakpoint was the centromeric area. The gene map reveals that the location of the genes for the epidermal growth factor receptor and growth control factor is on 7p and that of the collagen type I to III on 7q (10); there could possibly be a relationship between MM, which preferentially originates in the skin, and these gene loci.

Another peculiar feature of chromosomal abnormalities in MM appears to be the frequent occurrence of rearrangements of the centromere or centromeric area. This includes, as mentioned before, isochromosomes for the chromosome arms 1q and 6p, as well as central fusion of arms of different chromosomes (Case 2, 1q and 7q; Case 3, 4q and 7p), an observation also made by earlier investigators (5, 12). The involvement of 5q in an isochromosome formation, as seen in our Case 4, had also been observed in 2 other cases (16, 18). That the observation of an i(6p) is not an isolated event becomes apparent from the findings of Muir and Gunz (12) and Quinn et al. (16), who reported its occurrence in 2 cases of MM.

Another conclusion which can be made from our findings is that the frequency of marker chromosomes within a metaphase is not random, as is clearly shown in Case 2, in which M1 and M3 were regularly found in 2 copies in each of the karyotypes, whereas the other markers were present in single copies only. This clear-cut pattern for most markers was also observed in Case 4 and could allow the conclusion that rearrangements which are found in duplicate possibly occur during early oncogenesis in cells with a nearly diploid karyotype, whereas markers which are found as single copies in cells with a tetraploid or near-triploid karyotype are most probably generated later, during the karyotypic evolution, with duplication of the original chromosome set. Near-triploid karyotypes which are very frequently observed in MM might be the product of continuous loss of autosomes, the latter being of minor value for specific tumor clones.

Based on chromosomal aberrations in MM, obtained on either direct preparations (1, 2, 5) or long-term cultures (11, 12, 16, 18), we find much confirmation for our suggestion of the nonrandom involvement of chromosomes 1, 6, and 7 (Chart 1).

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Chromosomes in Melanoma


Fig. 1. Representative karyotype of Case 1, characterized by a hyperdiploid mode of 50 chromosomes. Markers involved chromosomes 1 to 4, 6, 7, 10, 12, 16, and 17. Marker M11 is not present in this particular cell.

Fig. 2. Karyotype of Case 2, with marker chromosomes M1 to M4. This karyotype was found to be in the triploid range, with a chromosome count of 72.

Fig. 3. Karyotype of Case 3, with a chromosomal number in the near-tetraploid range. The karyotype was tetrasomic for chromosomes 2, 8, 9, 16, 17, and 19. A detailed description of marker chromosomes M1 to M4 can be found in “Results.”

Fig. 4. Representative karyotype of Case 4, with marker chromosomes M1 to M5. Several autosomes (chromosomes 17, 20, and 21) were found to be tetrasomic, and chromosome 8 was found to be quintasomic. This metaphase contains 64 chromosomes.

Fig. 5. Marker chromosomes involving chromosomes 1, 6, and 7, found in Cases 1 to 4.
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