Cytogenetics of human malignant melanoma

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Abstract

There has been a tremendous recent resurgence of interest in examining chromosomal abnormalities in human cancers (particularly solid tumors). This interest has been stimulated by the molecular examination of recurring chromosome abnormalities, and the recognition that they may pinpoint the location of growth regulatory sequences (e.g. cellular oncogenes). This finding coupled with the clear recognition that specific chromosome abnormalities can also have important diagnostic and prognostic implications, have caused this avenue of research to expand at a significant rate. The following brief review will summarize the current state of knowledge regarding recurring chromosome abnormalities in human malignant melanoma. A discussion of chromosome changes in pre-malignant skin lesions, primary melanoma, and metastatic melanoma is described. Brief descriptions of the potential clinical utility, and biologic relevance of chromosome abnormalities in this disorder are also discussed.

Introduction

Based upon epidemiologic grounds there has been a significant increase in the incidence of malignant melanoma worldwide [1]. In North America a doubling of the incidence rate for melanoma has occurred each decade for the past forty years, and the current estimation is that > 1/100 Americans will develop melanoma by the year 2000 [1]. These alarming statistics coupled with the realization that three fourths of all deaths from skin cancer result from melanoma strongly suggests that significant attention should be focused on understanding not just the clinical behavior, but also the biology and genetics of this disorder. In this brief review, emphasis will be exclusively placed upon a discussion of the current state of knowledge of chromosomal alterations in this important neoplasm. However, the underlying viewpoint is that a proportion of these chromosomal changes will in fact represent byproducts of molecular events which themselves may play a causative role in melanoma carcinogenesis.

General overview

Cytogenetic examination of human cancers has significantly expanded over the past decade with more than 14,000 cases now reported in the world literature [2]. However, our knowledge base is weighted heavily toward human hematopoetic malignancies, with fewer than 20% of studies focusing on human solid tumors (which represent 80% of the cancers of man) [2, 3]. Nevertheless, the identification of recurring sites of chromosome change in human solid tumors (including melanoma) is increasing at a tremendous rate. The following sections will briefly discuss chromosomal alterations in malignant melanoma. A brief discussion of chromosome alterations in premalignant nevi and early stage melanomas will be presented first, followed by a

discussion of chromosomal changes occurring in metastatic disease. Finally discussion of very preliminary work suggesting that chromosomal abnormalities may correlate with clinical outcome in malignant melanoma, as well as studies related to the biologic significance of chromosome abnormalities will be presented.

Cytogenetic analysis of premalignant nevi and primary melanoma

The recognition of recurring sites of chromosome change has pinpointed several genes involved in cellular transformation. Accordingly, an examination of the earliest stages of tumor development, or the study of premalignant precursor lesions may provide significant information of biologic relevance. Unfortunately, there have been few reports on the cytogenetics of early malanocytic lesions, either common nevi, dysplastic nevi, or early (radial growth phase) primary melanomas. The studies undertaken to date have focused on two different approaches, first analyzing the constitutional karyotypes of patients with dysplastic nevus syndrome (DNS) [4, 5], or familial atypical multiple-mole melanoma (FAMMM) [6]. Second, cytogenetic examination of melanocytes from dysplastic nevi and primary melanoma.

Cytogenetic studies of peripheral blood lymphocytes from DNS or FAMMM patients have been undertaken because both represent inherited disorders characterized by the occurrence of multiple atypical moles and a strongly increased risk for melanoma. The mode of inheritance appears to be autosomal dominate for both disorders, although there is a considerable degree of non-penetrance. Chromosome analyses on both DNS and FAMMM patient populations have suggested either normal karotypes with normal spontaneous sister chromatid exchange rates in fibroblasts from these individuals, or slightly enhanced numbers of nonclonal cytogenetic abnormalities in affected versus control patients [4, 5, 7]. Specifically, some studies have shown a statistically significant increase in major structural abnormalities in the peripheral blood lymphocytes of melanoma patients, while

numeric abnormalities (again non-clonal and random) are observed more frequently in patients with DNS. Unfortunately, because no clonal cytogenetic abnormalities have yet been observed in any family with a hereditary disorder predisposing the melanoma, the results of generalized chromosome instability are difficult to interpret. Nevertheless, there does appear to be some slight increase in the level of spontaneous structural and numeric chromosome abnormalities in patients predisposed to the development of malignant melanoma.

In contrast to the cytogenetic study of peripheral blood from a reasonably large number of melanoma prone families, relatively few studies have focused on cytogenetic analysis of melanocytes from premalignant nevi and primary melanomas [8–10]. To date the concensus appears to be that chromosome abnormalities do exist in early stages of melanocytic neoplasia, and of interest the results are surprisingly non-overlapping between abnormalities observed in later stage melanomas. Parmiter et al. [9] studied a series of melanotic tumors including common nevi through metastatic melanomas and their data supported the view that terminal region of chromosome 10q (bands q24–q26) may harbor one or more genes involved in the early stages of melanocytic neoplasia. In contrast, Cowan et al. [8] analyzed cultures from a variety of lesions, including congenital and dysplastic nevi, primary melanoma and metastatic melanoma with results indicating that chromosome 9 (especially band region 9pter–p22) may be the site of a gene(s) important in the early steps of malignant transformation of melanocytes. In the study of Cowan [8], 22/26 cultures derived from dysplastic nevi had totally normal karyotypes, while 100% of cultures from primary and metastatic melanomas showed chromosome abnormalities. Chromosome 9 as well as chromosome 11 were the only two chromosomes altered in common between nevi and abnormal karyotypes of the malignant melanomas examined. In another study by McDonagh et al. [10], a patient with an 11p-constitutional abnormality was reported who developed dysplastic nevi at an early age.

These limited but provocative studies make up a selected, but representative base of knowledge regarding cytogenetic analysis from premalignant
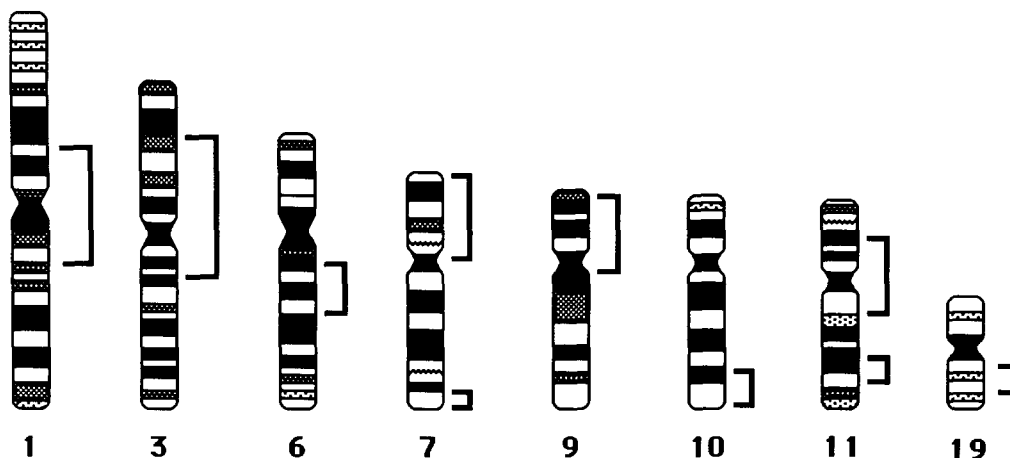
*Fig. 1.*Ideograms of human chromosomes most frequently altered in human malignant melanoma. The brackets indicate the most frequently altered band regions.

melanomas. To summarize, results to date suggest that patients with a hereditary predisposition for melanoma formation may show slightly increased levels of spontaneous chromosome abnormalities in both peripheral blood lymphocytes and human skin fibroblasts. Although, direct cytogenetic examination of dysplastic nevi and primary melanomas are still based on very small patient numbers, the results clearly suggest that chromosomes 9q and 10q may be important sites of chromosome change involved early in the genesis of this disorder. The gene(s) which may be disregulated by these chromosome abnormalities are not identified. However Cowan *et al.* [8] has suggested that the interferon gene cluster which maps to chromosome 9p [11] may be a possible target for the 9q chromosome rearrangements.

Chromosome abnormalities in metastatic malignant melanoma

A number of studies have appeared characterizing chromosomal alterations in metastatic melanoma [12–24]. Unfortunately, several variables exist in regards to the cultured cells so many of these reports have involved studies of established melanoma cell lines, and even studies of primary outgrowths have included a number of variables in technical procedures (e.g. days in culture, culture

media, chromosome harvesting techniques, etc.). However, despite these variables it is surprising that a general pattern of cytogenetic alteration in metastatic melanoma is beginning to emerge. First, $\geq 95\%$ of melanomas studied from primary outgrowths have recognizable chromosomal abnormalities, and the modal chromosome number is consistently in the near-triploid range. For example, a recent study from this laboratory [19], the modal chromosome number of 27 patients was 61 chromosomes/cell, with modal numbers ranging from ~ 40–90 chromosomes/cell.

Within this framework of multiple chromosomal rearrangements a number of chromosomes appear to be non-randomly involved in structural rearrangements including: 1, 3, 6, 7, 9, 10, 11 and 19.

Table 1 provides a listing of those recurring chromosomal alterations currently recognized in malignant melanoma by the Committee on Structural Chromosomal Changes in Neoplasia of the Human Gene Mapping (HMG) Workshop [3]. This listing recognizes only those abnormalities which have been reported by more than one laboratory, and in sufficient numbers to meet the criteria for HMG10.5 [3]. In addition to these recognizable changes melanomas are often characterized by the presence of unidentifiable markers (umars), which are a feature of many solid tumors and occur in $\sim 60\%$ of metastatic melanomas studied.

One feature of the chromosome abnormalities of

melanoma which is common to other solid tumors is the finding that chromosome alterations often characterize a chromosome band region, rather than a specific unique translocation (e.g. the t(9;22) translocation characterizing chronic myelogenous leukemia). Accordingly, as illustrated in Table 1, deletions of varying size (or translocations with various autosomal partners) may pinpoint a 'region' most frequently altered, rather than describe a single specific chromosome alteration. Figure 1 provides pictorial documentation of the band regions along chromosomes 1, 3, 6, 7, 9, 10, 11 and 19 most frequently altered in this disorder.

Chromosome 1 alterations are without question the most frequent site of chromosome change in metastatic melanoma with up to 80% of tumors displaying numeric or structural alterations (particularly flanking the centromere). Nevertheless, chromosome 1 is the single most frequently altered chromosome in virtually every solid tumor, so the biologic significance (and tumor specificity) of these changes is currently indeterminate. Of interest, Nowell and colleagues [18] have described a nonreciprocal translocation involving chromosomes 1 and 19 which appears to describe a subset of patients with this disease [t(1;19)(q12;p13)]. The region involved in chromosome 1 is an area of constitutive heterochromatin (thus with little if any genic activity) while the region of chromosome 19 involved in this translocation apparently lies close to (but not involving) the chromosomal loci of the insulin receptor gene (INSR) [18].

Chromosome 6 (both the long and short arm) is also very frequently altered in metastatic melanoma, with the most common alteration a simple deletion of the long arm (6q-). The region which appears to be most consistently lost surrounds bands 6q21. In addition to chromosome 6 deletions, the involvement of chromosome 6 in a series of nonreciprocal translocations (primarily with chromosome 1 – see Table 1), is also a relatively frequent finding in this disorder [20]. This translocation [t(1;6)(q11–12;q15–21)], has been observed in both established melanoma cell lines and direct cultures, and interestingly the region on chromosome 1 involved in this translocation is identical to that involved in a translocation with

chromosome 19 [18]. Although the site of the translocation on chromosome 6 (q12–13) is removed from that region most frequently involved in simple deletions (6q21), we have suggested that these alterations may in fact may both be related [20]. Specifically, the t(1;6) is a nonreciprocal translocation which in fact leads to the loss of sequences distal to 6q11. Thus, simple deletions as well as this nonreciprocal translocation both effectively remove sequences on 6q. Southern blotting using a panel of polymorphic DNA probes for chromoso-

Table 1. List of chromosome aberrations in malignant melanoma*

Region or band	Type of aberration	Status*	References
1p22–p11	Deletions, translocations	I	31
			12
			15
			16
			18
1q11–q12	Translocations, duplications	I	32
			12
			16
			18
1q11–q12	t(1;6)(q11–12;q15–21)	I	30
			20
6p11–q11	i(6p), translocations	II	31
			12
			15
6q11–q27	Deletions, translocations	I	13
			12
			7
			15
			17
			18
			16
6q15–q23	t(1;6)(q11–12;q15–21)	I	30
			20
7q11	Translocation	II	31
			12
			18
			16

mal 6q has recently demonstrated a high percentage of loss of 6q alleles in the tumor cells of patients with metastatic melanoma [25]. The possible biologic relevance of this loss of chromosome 6q sequences in malignant melanoma will be described later in the text.

Additional chromosomes which have been shown to be altered in metastatic melanoma include chromosome 7 and 11. Chromosome 7 alterations most frequently involve the short arm (7p) and it is of interest that enhanced expression of the epidermal growth factor receptor (EGFR) gene (which maps to 7p12) is associated with an increased number of copies of chromosome 7p in early stage melanomas [26]. Chromosome 11 abnormalities are more evenly divided between the short arm (particularly the terminal region) and the near distal long arm. Mitelman and colleagues [23] in a study of six cell lines from three melanoma patients have suggested that chromosome 11p alterations may play a role in melanoma tumorigenesis. The observation of 11p abnormalities in melanoma may relate somehow to the now well described presence of suppressor gene activity at 11p13 [27]. However, studies of malignant melanoma examining the loss of sequences on 11p using polymorphic probes has provided little evidence supporting the frequent loss of DNA within this chromosomal region [25, 28, 29].

Expression of silver stained nucleolar organizing regions (Ag-NORs) in human cancer

In normal human cells the chromosomal regions associated with nucleolar formation (NORs) are present on the short arms of the acrocentric (D- and G-group of chromosomes) [33]. Simple histochemical techniques for selectively staining active NORs have been developed and can be used to cytologically evaluate the location and number of transcriptionally active NORs [34, 35]. Malignant tissue (in contrast to normal cells) *in vivo* often display cytologic irregularies in number, size and shape of nuclei. Accordingly, studies have appeared for several years comparing the cytologic expression of these ribosomal cistrons in tumor and

normal tissue [36]. Studies of AgNORs in benign, dysplastic, and malignant melanocytic skin lesions have been performed by several laboratories [37–40]. The results of Howat *et al.* [37] illustrate the general findings in melanoma for this procedure. These investigators applied the AgNOR technique to 30 benign nevi, 30 dysplastic nevi and 30 malignant melanomas. AgNORs in 200 nuclei were scored and the means calculated. A significant difference was shown to exist between the number of AgNORs per nucleus for malignant melanomas versus those for benign nevi or dysplastic nevi. Additionally, there was no statistically significant difference between benign and dysplastic nevi in terms of the total number of NORs per cell. The problem with this and related studies relates to a significant overlap in counts between benign and dysplastic tissue, leading to a limiting in the diagnostic potential of this procedure. Thus, the authors have suggested the AgNOR technique may prove to be of value in helping to differentiate malignant melanoma from dysplastic nevi, although it will be unlikely to be of help in separating dysplastic from benign nevi. While this method is still a research tool, it appears to be gaining some support as an adjunct to routine diagnostic histopathology.

Relationship of cytogenetic abnormalities and clinical outcome in metastatic melanoma

Although, the value of chromosomal analysis is well established in hematopoietic malignancies, the relationship between chromosome abnormalities and clinical outcome in solid tumors has received relatively little study. Recently a study was performed in order to determine the distribution of clonal chromosomal abnormalities in patients with metastatic melanoma, and to correlate these abnormal karyotypes with clinical information [41]. These very preliminary analyses revealed that selected cytogenetic abnormalities were in fact predictive of clinical outcome [41].

Sixty two patients were entered in this study with 46 (74%) having sufficient metaphases for study. Ninety three percent of these patients had abnor-
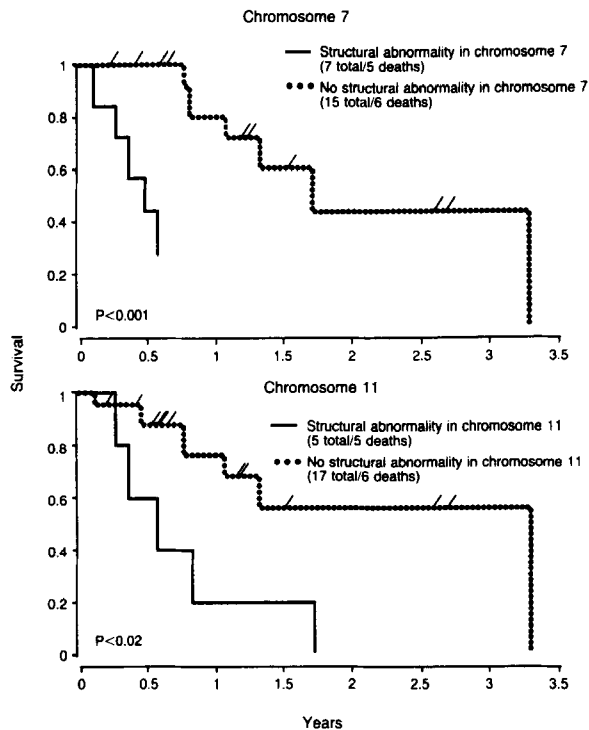
Fig. 2. Survival curves among patients who had stage III malignant melanoma with and without abnormalities in chromosomes 7 and 11. Diagonal lines intersecting survival curves denote patients for whom data were censored (used with permission).

mal karyotypes. There was no correlation between the presence or absence of normal mitoses and death rate ($P = 0.89$).

We also examined the representativeness of our patient population, and the effect of considering particular subsets of patients in our analyses. Additionally results were examined for patients whose tissue samples were analyzed cytogenetically to determine whether survival differed from those patients for whom cytogenetic analysis could not be performed (e.g. no growth, no metaphases, etc.). The results of these studies indicated that none of the clinical or demographic features examined differed significantly [41].

When specific chromosome abnormalities were examined for their association with clinical outcome patients with a structural abnormality of chromosome 7 or 11 had a significantly shorter survival duration ($p < 0.001$ and $p < 0.02$, respectively) than patients without such chromosome ab-

normalities (Fig. 2). This was true when survival was analyzed from the interval from cytogenetic study to death as well as the interval from diagnosis to death. Other karyotypic abnormalities examined (including those involving chromosomes 1, 3, 6, 9 and 21) did not correlate significantly with a shorter survival period.

Finally, although the numbers involved in our study were clearly insufficient to support a definitive multivariant analysis, we did perform analyses of the relation of structural abnormalities of chromosome 7 and 11 to survival from biopsy, diagnosis and the occurrence of stage 3 disease until death; with the variables including age, sex, nodal involvement, the presence of visceral metastases, and stage 3 disease in a proportional-hazards model with an underlying Weibull survival distribution. The results of the univariant analysis (with regard to the relation of these structural abnormalities) remained significant when subjected to multivariant analyses [41].

In summary, this aforementioned report on karyotypic analyses on patients with metastatic melanoma examined the relation between banded chromosomal analyses and clinical outcome of disease for this important neoplasm. The results indicated that shortened survival duration was associated with patients with structural abnormalities of chromosome 7 and 11. Although the abnormalities involved in these chromosomes were diverse, the region of each chromosome was surprisingly consistent. For example the rearrangements of chromosome 7 almost exclusively involved the short arm (25/27 cases). Similarly there was a clustering of chromosomal breakpoints along chromosome 11, which were about equally divided between the short arm (p11–p14) and the long arm (q23–q25). The underlying biology related to the observed difference in survival is clearly not understood. Several cellular oncogenes map to chromosome 7 and 11 but the influence of these oncogenes (if any) on the negative prognostic effects of alterations of chromosome 7 or 11 is unclear.

Finally, because of the large number of comparisons which were necessitated by the study design and the number of individual chromosomes examined, these results clearly require corroboration.

Nevertheless, in as much as cytogenetic analysis is providing useful information about hematopoietic malignancies, it appears likely that analysis of solid tumors (such as melanoma) will prove to be of similar value.

Biologic relevance of chromosome abnormalities in malignant melanoma

As mentioned previously molecular examination of specific chromosomal abnormalities has pinpointed the site of genes important in the process of growth regulation. Because malignant transformation is envisioned as a multistep process [42], recent studies highlighting the importance of tumor suppressor genes in the development of several human cancers are particularly of interest [43]. The supporting evidence for tumor suppressor genes is based upon a variety of studies including: 1) cytogenetic evidence for deletion of specific chromosomal regions, 2) loss of heterozygosity examined by RFLP analysis, and 3) study of somatic cell fusion experiments. The following section will briefly document an experimental approach using somatic cell fusion to assign a potentially important gene for malignant melanoma to human chromosome 6.

As mentioned previously chromosome banding analysis of human malignant melanoma has documented the nonrandom alteration of chromosome 6 (particularly the deletion of the long arm). In order to determine the relevance of chromosome 6 abnormalities in melanoma, a normal chromosome 6 was directly introduced into melanoma cell lines [44]. In these studies a human-mouse microcell hybrid was generated that contained a single copy of chromosome 6 (derived from a normal human diploid fibroblast) as its only human component.

Table 2[a]. Characteristics of the parental and microcell line

Cell line I.D.	Transformed phenotype (yes/no)	Growth in G418 (+/−)	Chromosome six introduced	No. tumors/ no. injected sites (day 10)	Soft agar cloning efficiency (%)	Cell generation time	HMB expression (+/−)
Parental cells							
Parental							
UACC-903	Yes	−		20/20	3.6	2.7	+++△
Parental							
UACC-091	Yes	−		12/12	5.4	2.0	+++
Microcell hybrid clones							
UACC-903							
MCH-361C1	No	+	6	0/12*	0.2	4.6	−−+
UACC-903							
MCH-361C1R•	Yes	−	−	6/6	N.D.	N.D.	+++
UACC-091							
MCH-360C1	No	+	6	0/12**	5.4	2.7	−−+
UACC-091							
MCH-360C4	No	+	6	0/12**	2.9	6.1	−−+
G418 resistant line							
UACC-903							
+psv₂neo	Yes	+		2/2	N.D.	N.D.	N.D.

△ Reactivity to HMB-45 was quantitated as uniformly positive [> 80% of all tumor cells postive (+++)] to focal reactivity [< 10% of tumor cells positive (−−+)] see Fig. 2.

• This revertant line was produced by culturing cells from a tumor formed following injection of MCH-361C1.

* Tumors arose in all animals after growth delay of up to 34 days. In every case examined, loss of the introduced 6 [assessed by cytogenetic and RFLP analysis (e.g. Fig. 3B)] occurred coincident with tumor formation.

** Following 60 days, 40% of animals developed tumors. However, loss of the introduced 6 was again confirmed in all cases by directly analyzing tumor tissue by RFLP analysis.

a Used with permission.

Fig. 3. Tumor formation 9 weeks following injection of 1×10^7 cells from a malignant melanoma cell line into the right flank of a nude (nu/nu) mouse. The identical number of cells from the (+ 6) microcell hybrid of this same cell line were injected at the same time into the left flank of this animal but were suppressed for tumor formation (see ref. 44).

The retention of chromosome 6 resulted from the insertion of the neomycin antibiotic resistance gene (psv$_2$neo) into this chromosome 6, and subsequent selection of microcell hybrids with medium containing the neomycin analog G418. This hybrid was then used to transfer an entire normal chromosome 6 into two different human melanoma cell lines via microcell transfer. A summary of characteristics of the parental and microcell line is presented in Table 2.

To summarize these results, the *in vitro* morphology of one of the two hybrid lines changed dramatically in response to the introduction of human chromosome 6. Both cell lines showed a diminution of melanoma associated antigen expression on (+ 6) microcell hybrids, one of the two microcell hybrids showed a reduction in soft agar clonogenicity, and both showed a lengthening of cell generation time. With this significant decline in melanoma associated antigen expression (together with the finding of altered cell morphology and diminished agar clonogenecity of (+ 6) microcell hybrids), a study was undertaken to determine whether the introduction of normal chromosome 6 would lead to suppression of tumor growth in nude mice.

After injection of cells $(5 \times 10^6 - 1 \times 10^7)$ from the parental cell lines and (+ 6) microcell hybrids into athymic (nu/nu) mice. Rapid growth occurred in 100% of animals from the parental lines within 5 to 7 days (Table 2, Fig. 3). For both tumor lines tested tumor formation was suppressed in animals receiving the (+ 6) when compared to the parental line. However, in several animals tumors developed following initial suppression, and the outgrowth from these (+ 6) microcell 'revertants' were subsequently shown to have lost the introduced chromosome 6 [44]. As a control for the introduction of chromosome 6 into the melanoma cell lines the psv$_2$neo gene was inserted by electroporation into melanoma cell lines and in contrast to the introduction of the chromosome 6, the addition of the selectable marker did not alter *in vitro* morphology or affect tumorigenicity.

These results have suggested that it may be reasonable to speculate that genetic information present on chromosome 6 can suppress the malignant phenotype of human melanoma cells. Following the suggestion of Vogelstein [45], tumor suppressor genes may have a hierarchial and incremental affect on the regulation of cell growth and our results may therefore suggest that this gene(s) on chromosome 6 may be acting early in the pathway of tumor formation.

Finally, because chromosome 1 is the most frequently altered chromosome in malignant melanoma, it was of significant interest when Bale *et al.* [46] recently mapped a gene for hereditary cutaneous malignant melanoma-dysplastic nevus to chromosome 1p using molecular genetic techniques and multipoint linkage. The authors speculated that this discovery could lead to a starting point for identifying a gene(s) for this disorder and eventually lead to a characterization of its function. However, the general significance of this finding is still unclear as other groups have failed to demonstrate linkage on 1p for families with this disorder, and further, studies from this same group of loss of alleles from the distal part of chromosome 1 appear to occur late (rather than early) in melanoma tu-

mor progression [47]. Nevertheless, this work is indeed of interest as a genetic component to the hereditary forms of this disease must certainly exist, and sequences on 1p may to play a role in the etiology of this disorder.

In summary then, on cytogenetic grounds the most highlighted karyotypic abnormalities in early melanomas involve chromosomes 9 and 10, while in metastatic melanoma these chromosomes are infrequently altered and instead there is the frequent involvement particularly of chromosomes 1 and 6. Further, the only chromosomal loci implicated in hereditary cutaneous malignant melanoma is chromosome 1p, but study of melanoma tumors appears to indicate that loss of sequences in this region are in fact late rather than early events in the development of this cancer. The conclusion then to be drawn is that much work remains to be done in order to understand the molecular basis for this important disorder. We should be prepared for surprises!

Conclusions

This review has provided a brief update on current progress in identifying recurring sites of chromosome change in human malignant melanoma. It is clear that additional work is required to more completely characterize this disorder, nevertheless several inferences can be made.

- Early stage melanomas and premelanotic lesions are characterized by minimal karyotypic alterations and the possible alteration of chromsomes 9p and 10q.
- Examination of metastatic melanoma implicates a subset of chromosomes in this disorder (particularly 1, 6, 7 and 11).
- Analysis of AgNORs in melanoma may provide a useful adjunct to routine histopathology in distinguishing overt melanotic lesions from dysplastic or benign nevi.
- Clinical correlations of chromosome change in malignant melanoma are very preliminary. By analogy to work in hematologic malignancies these studies may in the future provide impor-

tant insights into chromosomal regions whose alteration relates to clinical outcome.
- Biologic implications of chromosomal abnormalities in melanoma are also in their infancy. However, evidence for suppressor gene activity on chromosome 6 has been obtained.
- Continued study of the basic genetics of malignant melanoma should add significantly to our understanding of the causation and progression of this disorder.

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