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Significance of the small subtelomeric area of chromosome 1 (1p36.3) in the progression of malignant melanoma: FISH deletion screening with YAC DNA probes

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Abstract The short arm of chromosome 1 (1p), especially the subtelomeric region of 1p36, is a common site for abnormalities in malignant melanoma of the skin. In a recent study nodular melanomas displayed deletions of 1p36 in an augmented percentage of cases. To evaluate the dimension of these deletions and to study their significance for the progression of malignant melanoma we analyzed seven melanoma cell lines, 32 primary tumors, and 32 metastatic tumors by fluorescence in situ hybridization with the DNA probe D1Z2 in 1p36.3 and eight YAC DNA probes hybridizing to 1p36, 1p32, 1p31, and 1p21. All cell lines, 91% of the metastatic tumors and 63% of nodular melanomas showed a deletion of 1p36.3. In the YAC hybridization experiments, the most frequent deletions were found in 1p36 in all cell lines, in 13% of nodular melanoma, and in 44% of metastatic tumors. Deletions in 1p36 were mostly confined to a rather small area near the locus *D1Z2*. The frequent occurrence of this deletion in melanomas with a high metastatic potential and the abundant accumulation of this deletion in metastasis point to genes located on 1p36, which might be of significance for the metastatic capability of malignant melanoma.

Key words FISH · YAC · Melanoma · 1p

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Introduction

Deletions of the short arm of chromosome 1 are among the most frequent aberrations in many solid tumors including neuroblastoma, breast cancer and malignant melanoma of the skin [11]. Although Dracopoli et al. [5] demonstrated a deletion especially in the subterminal region of 1p, cytogenetic analysis and comparative genomic hybridization analysis (CGH) found chromosomal loss in all parts of 1p [25, 32] or a broader range of deletions from 1pter-1p33 [37]. More recently, loss of heterozygosity (LOH) has been detected in one locus in 1p36 [4]. In our recent fluorescence in situ hybridization (FISH) studies, we were able to demonstrate deletions with a subtelomeric DNA probe hybridizing at the locus *D1Z2* (1p36.3) in a surprisingly high percentage of nodular melanoma samples [28, 29]. These tumors represent melanomas with a high capacity to metastasize. However, in none of the studies mentioned above has the exact extension of the deletion been determined, so that the suggested involvement of certain candidate tumor suppressor genes, such as *p73* [15] remains speculative. To evaluate the real dimension of the deletion we performed fluorescence in situ hybridization (FISH) with different YAC clones spanning the regions 1p36, 1p32, 1p31 and 1p21. This is a reliable method of determining small deletions, which can easily be performed even on paraffin-embedded fixed material [7, 8] and is much more sensitive than conventional banding analysis [38]. To study the importance of this deletion in the progression of malignant melanoma we included in our study seven cell lines, 16 superficial spreading melanomas (SSM), 16 nodular melanomas (NM), and 32 metastases, which can be considered as the final step in the progression of malignant melanoma.

Materials and methods

Cell lines and tumor specimens

The M19 cell line was kindly provided by Dr. K. Schallreuther and Dr. N. Hibberts (Clinical and Experimental Dermatology, Uni-

versity of Bradford, Bradford, UK) and the cell lines DX3 [1], HMB2, MEWO and VUP [31], LT5.1 and T8 were generously provided by Dr. S. Burchill (ICRF Cancer Medicine Research Unit, St. James University Hospital, Leeds, UK). All cell lines were derived from nodular or metastatic melanomas. The 32 primary tumor samples consisted of different types of melanoma (16 NM and 16 SSM). In addition, 32 metastatic tumors from skin, lymph node or brain were included in the study. Histological diagnosis of malignant melanoma was documented for all cases. All specimens were subjected to additional independent histopathological review (C.W.). SSM were defined by the presence of a microinvasive or in situ radial growth phase that was composed of epithelioid neoplastic melanocytes arranged in a pagetoid pattern within the epidermis. A vertical-phase tumorigenic compartment-like tumor nodule formation may or may not have been present. Only SSM with vertical growth properties are considered to have metastatic potential, and these must be classed almost without exception in a higher tumor stage (measured as tumor thickness or level of invasion). The tumor was designated NM if it showed expansive preferential growth within the epidermis with tumor nodule formation and was not associated with the growth properties described for SSM. A metastasis was regarded as an accumulation of neoplastic melanocytes in lymph nodes and other organs in a patient in whom a melanoma with metastatic potential had been previously present.

Metaphase preparation of the cell lines

The cells were grown in RPMI with 10% fetal calf serum, penicillin and streptomycin for 8–10 days and incubated with Colcemid for 2–6 h (modified from Siracky et al.) [31]. Then the cells were collected and the metaphase spreads were prepared according to Rooney and Czepulowsky [30]. The chromosomes were spread on slides and dried overnight at 37°C.

Isolation of cells from paraffin-embedded sections

The isolation of the cells was done as previously described [28]. Briefly, an enzymatic digestion was carried out with collagenase and protease. The isolated cells were suspended in phosphate-buffered saline (PBS) with 5% BSA and dropped onto slides. The slides were air-dried and stored at –80°C until use.

YAC DNA probes

We used the YAC clones 783H7 and 956G5 (1p36), 406H3 and 928A10 (1p32, not overlapping [12]), 965F9, 835B8 and 963G1 (1p31, only short overlapping regions [13]) and 968G8 (1p21) from the CEPH mega YAC library (RZPD, Berlin, Germany). Corresponding STS and the localization of the YAC clones are shown in Fig. 1. Additional information on these YAC clones may be obtained with the CEPH Quickmap Infoclone available via the Internet (http://www.cephb.fr/bio/infoclone/html). The YAC DNA probes were prepared as described elsewhere [27].

FISH analysis

The metaphase spreads were pretreated with pepsin according to Tiainen et al. [33] and dehydrated. The slides with the isolated cells were pretreated as described elsewhere [28]. The cohybridization of the digoxigenin-labeled subtelomeric probe D1Z2 and the biotinylated centromeric probe D1Z1 (both Appligene Oncor, Germany) was done according to the manufacturer's instructions. In situ hybridization with the YAC DNA probes was performed as published before [27] with slight modifications. Briefly, a cohybridization of the biotinylated YAC probe and the digoxigenin-labeled centromeric probe D1Z1 (Appligene; prepared according to the manufactures instructions) was carried out. The probes were visualized with FITC-conjugated avidin (green fluorochrome) for the biotin-labeled probes and with Cy3-conjugated anti-digoxigenin antibody (red fluorochrome) for the digoxigenin-labeled centromeric or subtelomeric probe. Cells were counterstained with DAPI (1 µg/ml 4,6-diamidino-2-phenylindoledi-hydrochloride, Sigma). Signals were visualized with a Zeiss Axiophot fluorescence microscope (Zeiss, Oberkochem, Germany) with filter sets 09 (FITC), 00 (Cy3) and 02 (DAPI) and digitized for documentation with the MacProbe System (PSI, Chester, UK). The FISH results were verified independently by a second observer (data not shown). No significant differences between the results of the first and the second observer occurred.

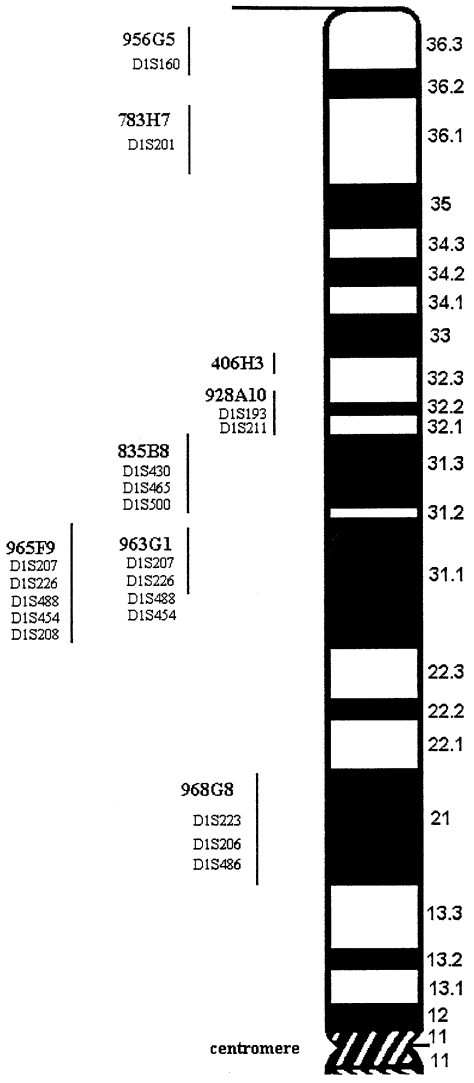


Fig. 1 Schematic picture of the short arm of chromosome 1. The numbers right to the chromosome correspond to the chromosomal bands. The names of the YAC clones (*bold numbers*) and matching STS are written to the *left* of the *ideogram*

Results

Control studies

Normal blood lymphocytes, fibroblasts and isolated cells from paraffin sections of normal tonsils and thyroid

Table 1 Results of the deletion screening by FISH (∘ no deletion, *d* deletions in more than 15% of cells, d^{50+} deletions in more than 50% of cells)

Cell lines	No. 1	D1Z2	1p36		1p32		1p31			1p21
			956G5	783H7	406H3	928A10	965F9	835B8	963G1	968G8
DX3	3–5	d^{50+}	d^{50+}	d^{50+}	∘	∘	d^{50+}	∘	∘	∘
HMB2	2–5	d^{50+}	d^{50+}	∘	∘	∘	∘	∘	∘	∘
LT5.1	2–5	d^{50+}	d^{50+}	∘	d^{50+}	∘	∘	d^{50+}	d^{50+}	∘
M19	3–6	d^{50+}	d^{50+}	∘	d^{50+}	∘	∘	∘	∘	∘
MEWO	3–4	d^{50+}	d^{50+}	d^{50+}	∘	∘	∘	∘	∘	∘
T8	3–6	d^{50+}	d^{50+}	∘	d^{50+}	d^{50+}	∘	∘	d^{50+}	∘
VUP	4–9	d^{50+}	d^{50+}	d^{50+}	∘	∘	∘	∘	∘	d^{50+}
Metastatic melanoma cases										
33	2–6	d	∘	∘	∘	∘	∘	∘	∘	∘
34	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
35	2–3	d	∘	∘	∘	∘	∘	∘	∘	d
36	2	d	∘	∘	∘	∘	∘	∘	∘	∘
37	2–4	d	d	∘	∘	∘	d	∘	∘	∘
38	2–3	d	∘	∘	∘	∘	∘	∘	∘	∘
39	3–4	d	d	d	∘	∘	∘	∘	∘	∘
40	2–5	d	d	∘	∘	d	∘	∘	∘	∘
41	2–4	d	∘	∘	∘	∘	∘	∘	∘	d
42	2–3	∘	∘	∘	∘	d	∘	d	d	∘
43	2–5	d	d	∘	∘	∘	∘	∘	∘	∘
44	2–3	d	d	∘	∘	∘	∘	d	∘	∘
45	2–4	d	d	∘	∘	∘	∘	∘	∘	∘
46	2–4	d	d	d	∘	∘	∘	d	∘	∘
47	2–6	d	∘	∘	∘	∘	d	d	∘	∘
48	4–6	d	∘	∘	∘	∘	∘	∘	∘	∘
49	4–7	d	∘	∘	∘	∘	d	d	∘	∘
50	2–3	∘	∘	∘	∘	d	∘	∘	∘	∘
51	2–5	d	d	d	∘	d	∘	∘	∘	∘
52	2–6	d	∘	∘	∘	d	∘	∘	∘	∘
53	2–3	d	d	∘	∘	∘	∘	∘	d	∘
54	4–6	d	∘	∘	∘	∘	∘	∘	∘	∘
55	2	d	d	d	∘	∘	∘	∘	∘	∘
56	2	d	∘	∘	∘	∘	∘	∘	∘	d
57	2–6	d	d	d	∘	∘	∘	∘	∘	∘
58	2–3	d	∘	∘	∘	∘	∘	∘	∘	∘
59	2–5	d	∘	∘	∘	d	∘	∘	∘	∘
60	2–3	d	∘	∘	d	∘	∘	∘	∘	∘
61	2–6	d	d	d	∘	∘	∘	∘	∘	∘
62	5–7	d	d	∘	∘	∘	d	∘	∘	∘
63	3–7	d	d	d	∘	∘	∘	∘	∘	∘
64	2–3	d	∘	∘	∘	∘	∘	∘	∘	d
Nodular melanoma cases										
17	2	∘	∘	∘	∘	∘	∘	∘	d	∘
18	2	d	∘	∘	∘	∘	∘	∘	∘	d
19	2	d	∘	∘	∘	∘	∘	∘	∘	∘
20	2–3	d	d	∘	∘	∘	∘	∘	∘	∘
21	2–4	∘	∘	∘	∘	∘	∘	∘	d	∘
22	2–5	∘	∘	∘	∘	∘	∘	∘	∘	∘
23	2–3	∘	∘	∘	d	∘	∘	∘	d	∘
24	2	d	∘	∘	∘	∘	∘	∘	∘	∘
25	2	d	∘	∘	∘	∘	∘	∘	∘	∘
26	2–4	d	∘	∘	∘	∘	∘	∘	∘	∘
27	2–3	d	∘	∘	∘	∘	∘	∘	d	∘
28	2–3	∘	∘	∘	∘	∘	∘	∘	∘	d
29	2–5	d	d	∘	∘	∘	∘	∘	∘	∘
30	2–4	d	∘	∘	∘	∘	∘	∘	∘	∘
31	2–3	d	∘	∘	∘	∘	∘	∘	∘	∘
32	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
Superficial spreading melanoma cases										
1	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
2	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
3	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
4	2–3	∘	∘	∘	∘	∘	∘	d	d	∘
5	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
6	2	∘	∘	∘	∘	∘	∘	∘	∘	∘

Table 1 Continued

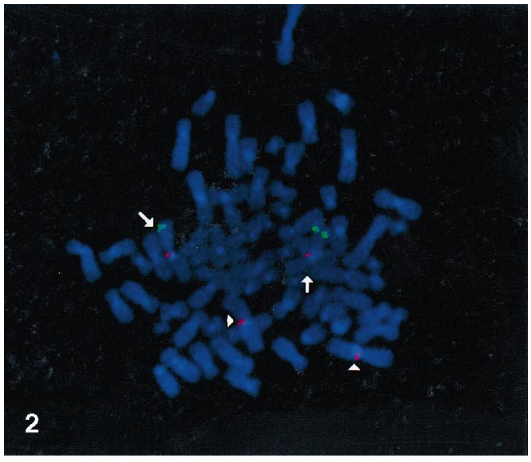
Cell lines	No. 1	D1Z2	1p36		1p32		1p31			1p21
			956G5	783H7	406H3	928A10	965F9	835B8	963G1	968G8
7	2	∘	∘	∘	d	∘	∘	∘	∘	∘
8	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
9	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
10	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
11	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
12	2–3	∘	∘	∘	∘	∘	∘	∘	∘	∘
13	2	∘	∘	∘	∘	∘	∘	∘	∘	∘
14	2–4	∘	∘	∘	∘	∘	∘	∘	∘	∘
15	2–4	∘	∘	∘	∘	∘	∘	∘	d	∘
16	2	∘	∘	∘	∘	∘	∘	∘	∘	∘

glands were used as controls. All used YAC DNA probes, the subtelomeric probe D1Z2, and the centromeric probe showed an expected number of signals in peripheral blood lymphocytes and fibroblasts of the skin from healthy donors. Single and cohybridization experiments were carried out. The frequency of a trisomy ranged between 0.2% and 0.9% (median 0.5%) and the frequency of a monosomy ranged between 4.5% and 9.7% (median 7.1%). We defined the cut-off level for a trisomy at 1% (median + 2 standard deviations) and for a monosomy at 10% (median + 2 standard deviations). At least 500 cells were analyzed for each donor and probe combination. The YACs were judged to be nonchimeric from this FISH analysis.

The frequency of a trisomy was lower than 1%, and the frequency of a monosomy was lower than 15% in isolated cells from paraffin sections of normal tonsils. The frequency of a trisomy ranged between 0.1% and 0.8% (median 0.45%) and the frequency of a monosomy, between 6.5% and 13.1% (median 9.2%). We defined the cut-off level for a trisomy at 1% (median + 2 standard deviations) and for a monosomy at 15% (median + 2 standard deviations). We analyzed 200–500 cells for each sample and probe combination.

FISH on melanoma cell lines and isolated cells from paraffin sections of melanomas

In the hybridization experiments, either a combination of one YAC DNA probe and the centromeric probe of chromosome 1 or a combination of the subtelomeric probe in 1p36 and the centromeric probe was used to allow direct evaluation of deletions in chromosome 1. The percentage of aberrant cells in the tumor samples varied between 52% and 88% (in NM and SSM on average 62%, in MT 78%). If a deletion was detectable, only one chromosome 1 remained normal, even in cells with three or more signals for the centromeric region of chromosome 1 (with the exception of the cell lines). The deletion was mostly restricted to one of the regions analyzed, YAC probes from 1p36 and 1p32 being deleted in the cell line T8 only. The results are summarized in Table 1.



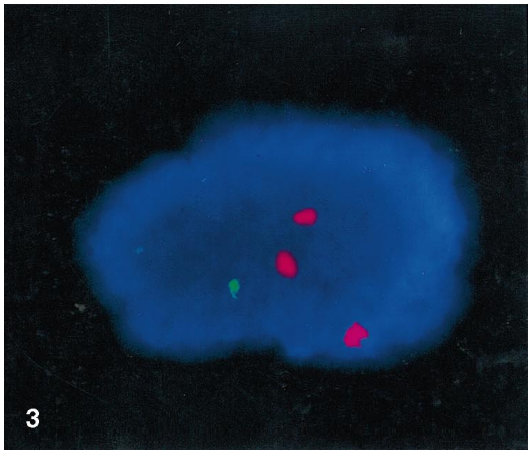


Fig. 2 Metaphase spread of the cell line DX3 after hybridization with the YAC DNA probe 783H7 in 1p36 (*green signals*) and the DNA probe D1Z1 (centromeric region of chromosome 1, *red signals*). The *arrows* point to the two normal chromosomes 1 (with one *red* and one *green signal*) and the *arrowheads* point to the two chromosomes 1 with a deletion in 1p36 (only a *red signal*)

Fig. 3 Isolated interphase cells of paraffin-embedded tissue from a nodular melanoma (case 27) after hybridization with the YAC DNA probe 963G1 in 1p31 (*green signals*) and D1Z1 (centromeric region of chromosome 1, *red signals*). The cell has three *red signals* (for three copies of chromosome 1), but only one *green signal* for the region 1p31

The highest frequency of deletions could be found in the cell lines. Not only the subtelomeric probe D1Z2 but also the YAC DNA probe 956G5 from 1p36 showed deletions in all cell lines. In three cell lines an additional deletion with 783H7 was demonstrable (Fig. 2). In addition, in three cell lines deletions in 1p31 occurred, in three cell lines deletions in 1p32, and in one cell line deletions in 1p21. In general, the results in the cell lines were rather heterogeneous and only deletions found in at least 50% of the analyzed cultured cells were mentioned.

Metastatic melanomas also showed a high percentage of deletions, especially of the locus *D1Z2* (91%) and the YAC DNA probe 956G5 hybridizing to 1p36 (44%). We were never able to demonstrate a deletion with this YAC DNA probe unless there was a deletion of the locus *D1Z2*. Furthermore, deletions of the more centromeric YAC DNA probe 783H7 occurred only in MT with deletions of the YAC 956G5. Deletions with the other YAC DNA probes were detected less frequently (in 1p32 in 22%, in 1p31 in 25% and in 1p21 in 13%).

In nodular melanoma, deletions other than in *D1Z2* were found in much lower percentages of cases. About half of the samples showed aberrations with the subtelomeric DNA probe, but deletions with the YAC DNA probes from 1p36 occurred only in 9%, deletions in 1p32 in only one sample, in 1p31 in 25% (Fig. 3), and in 1p21 in 13%. In SSM few deletions were detectable at all – and none at all in 1p36. Deletions in 1p31 were seen in 13% of samples and deletions in 1p32 in 6%.

Discussion

Investigators working on many solid tumors have found the short arm of chromosome 1 an interesting region. Deletions of this chromosomal region have been detected in tumors of the nervous system, lung cancer, breast cancer, and germ cell tumors, and also in malignant melanoma of the skin [10, 11, 14]. Especially the subtelomeric region around the locus *D1Z2* has proved to be a major site of rearrangements in these tumors. The importance of this region has been underlined by the finding of such candidate tumor suppressor genes as *p73* [15], the human Kruppel-related 3 zinc finger gene [21], and PITSLRE [17], or genes involved in cell division control, such as the *p58* protein kinase [6] and apoptosis (DFFA, [18]) in 1p36. One or a number of these genes may be involved in the pathogenesis of one or all of the solid tumors mentioned above.

Up to 60% of melanomas showed rearrangements of the whole of chromosome 1 according to Heim and Mitelman [11]. In previous FISH studies of malignant melanomas, however, our results show a higher frequency of aberrations at chromosome 1. We found a subtelomeric deletion in 1p36 with the repetitive DNA probe D1Z2 in 60% of nodular melanomas [29] and in 88% of metastatic melanomas [28]. To investigate the importance and the dimension of the deletion in 1p36, we used two YAC DNA probes of 1p36, one of them hybridizing in the proximal neighborhood of *D1Z2* and the other more centromeric, and six other YAC clones from 1p32, 1p31 and 1p21 to determine the deletion status of malignant melanoma.

We were able to demonstrate deletions of all investigated regions in all analyzed tumors samples. In general, deletions in 1p36 were the most frequent, and loss of chromosomal material in 1p21 occurred only rarely. With increasingly malignant behavior in melanoma from SSM through NM and to metastases, which are the final step in tumor progression, the frequency of deletions also increased. This is in accord with the results of Dracopoli et al. [5] and Parmiter and Novell [26] for malignant melanoma of the skin, although in other solid tumors this deletion has also been connected with the onset of tumor development [3]. Since the deletion in 1p36 occurs in nearly all metastases and even in metastatic tumors derived from SSM, while the tumor of origin never showed this aberration, it could well reflect an important event in the development of metastatic behavior of melanoma cells [34].

We were able to demonstrate that loss of chromosomal material in 1p36 is mostly restricted to a rather small region between the locus D1Z2 and the region of the STS D1S160. This finding increases the possibility that one of the above candidate tumor suppressor genes is affected by the deletion, because the location of the gene *p73* has been defined recently as between D1Z2 and D1S47 [20] – a region that is covered by the YAC clone 956G5.

Deletions in 1p31 in malignant melanoma have been described rather seldom so far in cytogenetic studies (3 out of 51 tumor samples by Thompson et al. [32]). Mostly, aberrations in 1p31 were part of greater deletions or translocations extending to 1p21 [36]. In this study, deletions seem to be restricted predominantly to 1p31, because we could not demonstrate a loss of signals from YAC probes 963G1 (or 835B8, 965F9) and 968G8 hybridizing to 1p21 in any of our cell lines or tumor samples. In addition, only in one cell line and three MT were all three YAC probes from 1p31 deleted, and in the majority of cells the deletion was limited to sequences detectable with 963G1. The difference between our results and those in the literature might be explained by the sensitivity of FISH detecting smaller deletions than conventional banding techniques. Dracopoli et al. demonstrated LOH in this region in 23% of samples analyzed, especially in metastatic melanoma [5]. LoH in 1p31 has also been found in other solid tumors, including breast cancer [13, 23], neuroblastoma [2] and male germ cell tumors [22]. Several genes have been reported to map on chromosome 1p31-p32, including the potential tumor suppressor genes VCAM-1 [24] and p18 [9].

Although aberrations concerning 1p21–p22 have frequently been reported in malignant melanoma [19, 33], the two other investigated regions in 1p21 and 1p32 played only a minor role in our study. This could have been due to the choice of the YAC clones. The region of interest could be smaller than the chosen YAC clone, or

the deleted sequence and the YAC probe might overlap only partly. FISH studies with other YAC clones and cosmid probes hybridizing in this region could provide more information about the deleted sequences in 1p21.

In conclusion, our results indicate that a deletion in 1p36 occurs more often than previously described, but it is restricted to a rather small region around the locus *D1Z2*. An involvement of the candidate tumor suppressor gene *p73* might be possible, although recent investigations of melanoma cell lines did not demonstrate any mutations of this gene [16, 35]. DNA sequencing analysis of primary tumor specimens should be done to find a definite answer to this question. Regardless of which gene or genes are lost due to this deletion, they could harbor functions relevant to the emergence of the metastatic capability of malignant melanoma.

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References

1. Albino AP, Lloyd KO, Houghton AN, Oettgen HF, Old LJ (1981) Heterogeneity in surface antigen and glycoprotein expression of cell lines derived from different melanoma metastases of the same patient. J Exp Med 154:1764–1778
2. Avigad S, Benyaminy H, Tamir Y, Luria D, Yaniv I, Stein J, Stark B, Zaizov R (1997) Prognostic relevance of genetic alterations in the p32 region of chromosome 1 in neuroblastoma. Eur J Cancer 33:1983–1985
3. Bardi G, Pandis N, Fenger C, Kronberg O, Bomme L, Heim S (1993) Deletion of 1p36 as a primary chromosomal aberration in intestinal tumorigenesis. Cancer Res 53:1895–1898
4. Böni R, Matt D, Voetmeyer A, Burg G, Zhuang Z (1998) Chromosomal allele loss in primary cutaneous melanoma is heterogeneous and correlates with proliferation. J Invest Dermatol 110:215–217
5. Dracopoli NC, Harnett P, Bale SJ, Stanger BZ, Tucker MA, Housman DE, Keffort RF (1989) Loss of alleles from the distal short arm of chromosome 1 occurs late in melanoma tumor progression. Proc Natl Acad Sci USA 86:4614–4618
6. Eipers PG, Barnoski BL, Han J, Carroll AJ, Kidd VJ (1991) Localization of the expressed human p58 protein kinase chromosomal gene to chromosome 1p36 and a highly related sequence to chromosome 15. Genomics 11:621–629
7. El-Naggar AK, van Dekken HD, Ensign LG, Pathak S (1994) Interphase cytogenetics in paraffin-embedded sections from renal cortical neoplasms. Cancer Genet Cytogenet 73:134–141
8. Ghaleb AH, Pizzolo JG, Melamed MR (1996) Aberrations of chromosomes 9 and 17 in bilharzial bladder cancer as detected by fluorescence in situ hybridization. Am J Clin Pathol 106: 234–241
9. Guan K-L, Jenkins CW, Li Y, Nichols MA, Wu Y, O'Keefe CL, Matera AG, Xiong Y (1994) Growth suppression by p18, a $p16^{INK4/MTS1}$- and $p14^{INK4B/MTS2}$-related CDK6 inhibitor, correlates with wild-type pRB function. Genes Dev 8:2939–2952
10. Hashimoto N, Ichikawa D, Arakawa Y, Date K, Ueda S, Nakagawa Y, Horii A, Nakamura Y, Abe T, Inazawa J (1995) Frequent deletions of material from chromosome arm 1p in oligodendroglial tumors revealed by double-target fluorescence in situ hybridization and microsatellite analysis. Genes Chromosom Cancer 14:295–300
11. Heim S, Mitelman F (1995) Cancer cytogenetics: chromosomal and molecular genetic aberrations of tumor cells, 2nd edn. Wiley-Liss, New York
12. Hellsten E, Vesa J, Heiskanen M, Mäkelä TP, Järvelä I, Cowell JK, Mead S, Alitalo K, Palotie A, Peltonen L (1995) Identification of YAC clones for human chromosome 1p32 and physical mapping of the infantile neuronal ceroid lipofuscinosis (INCL) locus. Genomics 25:404–412
13. Hoggard N, Hey Y, Brintnell B, James L, Jones D, Mitchell E, Weissenbach J, Varley JM (1995) Identification and cloning in yeast artificial chromosomes of a region of elevated loss of heterozygosity on chromosome 1p31.1 in human breast cancer. Genomics 30:233–243
14. Jenderny J, Köster E, Borchers O, Meyer A, Grote W, Harms D, Jänig U (1995) Detection of chromosome aberrations in paraffin sections of seven yolk sac tumors of childhood. Hum Genet 96:644–650
15. Kaghad M, Bonnet H, Yang A, Creancier L, Biscan J-C, Valent A, Minty A, Chalon P, Lelias J-M, Dumont X, Ferrara P, McKeon F, Caput D (1997) Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 90:809–819
16. Kroiss MM, Bosserhoff AK, Vogt T, Buettner R, Bogenrieder T, Landthaler M, Stolz W (1998) Loss of expression or mutations in the p73 tumour suppressor gene are not involved in the pathogenesis of malignant melanoma. Melanoma Res 8:504–509
17. Lahti JM, Valentine M, Xiang J, Jones B, Amann J, Grenet J, Richmond G, Look AT, Kidd VJ (1994) Alterations in the PITSLRE protein kinase gene complex an chromosome 1p36 in childhood neuroblastoma. Nat Genet 7:370–375
18. Leek JP, Carr IM, Bell SM, Markham AF, Lench NJ (1997) Assignment of the DNA fragmentation factor gene (DFFA) to human chromosome bands 1p36.3->p36.2 by in situ hybridization. Cytogenet Cell Genet 79:212–213
19. Limon J, Dal Cin P, Sait SN, Karakousis C, Sandberg AA (1988) Chromosome changes in metastatic human melanoma. Cancer Genet Cytogenet 30:201–211
20. Lo Cunsolo C, Casciano I, Banelli B, Tonini GP, Romani M (1998) Refined chromosomal localization of the putative tumor suppressor gene TP73. Cytogenet Cell Genet 82:199–201
21. Maris JM, Jensen J, Sulman EP, Beltinger CP, Allen C, Biegel JA, Brodeur GM, White PS (1997) Human Kruppel-related 3 (HRK3): a candidate for the 1p36 neuroblastoma tumour suppressor gene? Eur J Cancer 33:1991–1996
22. Mathew S, Murty VV, Bosl GJ, Chaganti RS (1994) Loss of heterozygosity identifies multiples sites of allelic deletions on chromosome 1 in human male germ cell tumors. Cancer Res 54:6265–6269
23. Nagai H, Negrini M, Carter SL, Gillum DR, Rosenberg AL, Schwartz GF, Croce CM (1995) Detection and cloning of a common region of loss of heterozygosity at chromosome 1p in breast cancer. Cancer Res 55:1752–1757
24. Osborn L, Hession C, Tizard R, Vasallo C, Luhowskyl S, Chi-Rosso G, Lobb R (1989) Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell 59:1203–1211
25. Ozisik YY, Meloni AM, Altungoz O, Peier A, Karakousis C, Leong SPL, Sandberg AA (1994) Cytogenetic findings in 21 malignant melanomas. Cancer Genet Cytogenet 77:69–73
26. Parmiter AH, Nowell PC (1993) Cytogenetics of melanocytic tumors. J Invest Dermatol 100:24S-258S
27. Poetsch M, Weber-Matthiesen K, Plendl H-J, Grote W, Schlegelberger B (1996) Detection of the t(14;18) chromosomal translocation by interphase cytogenetics with YAC probes in follicular lymphoma and nonneoplastic lymphoproliferation. J Clin Oncol 14:963–969
28. Poetsch M, Woenckhaus C, Dittberner T, Pambor M, Lorenz G, Herrmann FH (1998) Increased frequency of numerical chromosomal abnormalities and 1p36 deletions in isolated cells of paraffin sections of malignant melanoma detected by interphase cytogenetics. Cancer Genet Cytogenet 104:146–152
29. Poetsch M, Woenckhaus C, Dittberner T, Pambor M, Lorenz G, Herrmann FH (1998) Differences in chromosomal aberrations between nodular and superficial spreading malignant melanoma detected by interphase cytogenetics. Lab Invest 78:883–888

30. Rooney DE, Czepulowsky BH (1992) Human cytogenetics: a practical approach, vol I. Oxford University Press, Oxford
31. Siracky J, Blasko M, Borovansky J, Kovarik J, Svec J, Vrba M (1982) Human melanoma cell lines: morphology, growth, and α-mannosidase characteristics. Neoplasma 29:661–668
32. Thompson FH, Emerson J, Olson S, Weinstein R, Leavitt SA, Leong SPL, Emerson S, Trent JM, Nelson MA, Salmon SE, Taetle R (1995) Cytogenetics of 158 patients with regional or disseminated melanoma. Cancer Genet Cytogenet 83:93–104
33. Tiainen M, Popp S, Parlier V, Emmerich P, Bellomo MJ, Ruutu T, Cremer T, Knuutila S (1992) Chromosomal in situ suppression hybridization immunologically classified mitotic cells in hematologic malignancies. Genes Chromosom Cancer 4:135–140
34. Trent JM, Meyskens FL, Salmon SE, Ryschon K, Leong SPL, Davis JR, McGee DL (1990) Relation of cytogenetic abnormalities and clinical outcome in metastatic melanoma. N Engl J Med 322:1508–1511
35. Tsao H, Zhang X, Majewski P, Haluska FG (1999) Mutational and expression analysis of the *p73* gene in melanoma cell lines. Cancer Res 59:172–174
36. Vance JM, Matise TC, Wooster R, Schutte BC, Bruns GAP, van Roy N, Brodeur GM, Tao YX, Gregory S, Weith A, Vaudin M, White P (1997) Report of the third international workshop on human chromosome 1 mapping 1997. Cytogenet Cell Genet 78:153–182
37. Wiltshire RN, Duray P, Bittner ML, Visakorpi T, Meltzer PS, Tuthill RJ, Liotta LA, Trent JM (1995) Direct visualization of the clonal progression of primary cutaneous melanoma: application of tissue microdissection and comparative genomic hybridization. Cancer Res 55:3954–3957
38. Zhang Y, Weber-Matthiesen K, Siebert R, Matthiesen P, Schlegelberger B (1997) Frequent deletions of 6q23–24 in B-cell non-Hodgkin's lymphomas detected by fluorescence in situ hybridization. Genes Chromosom Cancer 18:310–313