

Establishment and Characterization of a Melanoma Cell Line from a Xeroderma Pigmentosum Patient: Activation of *N-ras* at a Potential Pyrimidine Dimer Site¹

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ABSTRACT

Patients suffering from the genetic disorder xeroderma pigmentosum (XP) display an extreme sensitivity of their skin to sun (UV) exposure and predisposition to skin cancer due to deficiencies in the excision DNA repair pathway. Here we describe the establishment and characterization of the first tumor cell line derived from an XP patient (belonging to complementation group C). The melanoma cell line designated XP44RO(Mel) has retained its tumorigenic and XP phenotype (UV sensitivity, reduced unscheduled DNA synthesis) and showed karyotypic abnormalities characteristic of melanomas. Transfection of XP44RO(Mel) DNA to NIH3T3 cells and oligonucleotide hybridization revealed that the *N-ras* oncogene was activated by an A·T to T·A or C·G transversion at the third position of codon 61. This mutation occurs at a dipyrimidine site. It is likely initiated by a UV-induced pyrimidine dimer and is of a type rarely observed in mammalian shuttle vector systems and endogenous genes after UV irradiation.

INTRODUCTION

One of the best characterized examples of the relationship between exposure to DNA-damaging agents and tumorigenesis is the human syndrome XP.³ This rare, autosomal recessive disorder is marked by extreme sensitivity of the skin to sun (UV) exposure and early onset of freckling. Frequently, ocular and neurological abnormalities are registered. There is predisposition to neoplasia (basal and squamous cell carcinomas and melanomas) almost exclusively restricted to sun-exposed areas of the skin (see Ref. 1 for a review on XP). The >1000-fold-elevated skin cancer incidence is thought to be an indirect consequence of defects in DNA repair processes. In most XP patients damage induced in DNA by UV light cannot be eliminated effectively, because of a deficiency in the excision repair pathway (2), one of the major repair routes in the cell (see Ref. 3 for a review on DNA repair). The biochemical complexity underlying this system is amply illustrated by the discovery of extensive genetic heterogeneity within excision deficient XP patients: thus far, nine complementation groups have been identified using cell fusion techniques (4). All of these appear to be disturbed in early (preincision) events of the excision process (5, 6). The precise molecular defect in any of these XP mutations is not understood.

To date no cell lines have been established from XP tumors nor have mutations been analyzed, which are implicated in tumor etiology and may have been the consequence of the repair deficiency in XP. Here we describe the isolation and characterization of a melanoma cell line from an XP individual assigned

to complementation group C. Cytogenetic analysis revealed chromosomal abnormalities not uncommon for malignant melanoma. The tumorigenic properties of the cells were related to an activated *N-ras* gene, that appeared to have undergone a critical base substitution in codon 61. This base substitution is observed at a site for potential pyrimidine dimer formation as a consequence of exposure to UV.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

Establishment of XP44RO(Mel). A testicular tumor was removed from a 31-year-old patient with XP. The pigmented tumor was diagnosed as malignant melanoma probably resulting from a metastasis of a primary skin tumor which has been excised 1 year before. Aside from the melanoma, the patient has been treated for various other skin tumors, including several basal cell carcinomas, and a squamous cell carcinoma. Part of the testis tumor was minced and small tissue fragments were placed in 2.5-cm plastic culture dishes. A thin layer of culture medium (a 1:1 mixture of Ham's F10 medium and DMEM, supplemented with 15% fetal calf serum and antibiotics) was added and the cultures were incubated at 37°C in a humidified atmosphere with 5% CO₂. After 2 days most of the tissue fragments had attached to the culture dish, allowing addition of more medium. After 5 days many small cells with spindle to dendritic shapes were growing out of the tissue fragments. These presumptive tumor cells increased rapidly in number and could be trypsinized and transferred to larger culture vessels within 2 weeks after setting up the culture. The cell line thus obtained was designated XP44RO(Mel). Besides tumor cells also fibroblasts grew out off the tissue fragments, permitting the establishment of a fibroblast cell strain XP44RO. Since some tissue fragments gave outgrowth of only one cell type, *i.e.*, either tumor cells or fibroblasts, the XP44RO and XP44RO(Mel) cultures were obtained without mutual contamination. In addition the tumor cell line was subcloned.

Other Cell Lines. The SV40-transformed cell line CW-12 (generously provided by Dr. R. E. Moses, Houston, TX) and the primary fibroblast strain XP21RO both from XP-C patients are described elsewhere (7, 8). MEL17RO is a melanoma cell line and C5RO a primary fibroblast established from repair-proficient individuals. All primary fibroblasts were routinely cultured in Ham's F10 medium (supplemented with 10% FCS and antibiotics), the SV40-transformed cells in a 1:1 mixture of F10/DMEM containing 5–10% FCS.

DNA-repair Assays

UV-survival Experiments. Cells in normal growth medium were inoculated into 10-cm Petri dishes at a density of 5×10^5 cells/dish. One day after seeding cells were irradiated with 254-nm UV from a low pressure mercury germicidal lamp (Philips TUV) at a dose rate of 0.6 J/m²s, trypsinized, counted, and inoculated into 6-cm Petri dishes at densities of 2×10^2 – 10^4 cells/dish. XP44RO(Mel) cells were seeded into dishes with lethally X-irradiated (4 krads) C5RO primary fibroblast cells as feeder layer. The cells were cultured for 7–14 days after which period the clones were fixed, stained with Giemsa's solution, and scored.

Unscheduled DNA Synthesis. After rinsing in PBS the cells on coverslips were UV-irradiated using a dose of 16 J/m². The cultures were subsequently incubated for 2 h in medium containing 10% dialyzed FCS and 10 μ Ci/ml [³H]thymidine (46 Ci/mM), rinsed in PBS, and fixed using Bouin's fixative (composition: fifteen parts pikric acid,

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³ The abbreviations used are: XP, xeroderma pigmentosum; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; PBS, phosphate buffered saline; Ig, immunoglobulin; UDS, unscheduled DNA synthesis.

five parts formalin, one part glacial acetic acid) for 5–15 min. The coverslips were mounted on slides and processed for autoradiography by Kodak AR10 stripping film. Exposure time was 1 week at 4°C. After development and fixation the cells were stained with Giemsa's solution. Grains in a fixed square of 30 nuclei were counted.

Transfection Protocols for Transformation and Induction of Tumorigenicity of NIH3T3 Cells

High molecular weight genomic DNA of XP44RO(Mel) (30 µg), in coprecipitate with plasmid pMCS (0.5 µg) (9), containing the neomycin resistance marker was transfected to mouse NIH3T3 cells ($4-5 \times 10^5$ cells/dish) using a modification of the calcium phosphate precipitation method developed by Graham and Van der Eb (10, 11). The precipitate remained on the cells for 17 h. In experiments to test transformants for tumorigenicity the transfected cell population of each dish was trypsinized 1 day after transfection and replated into six Petri dishes. 48 h after transfection the culture medium was replaced by medium containing 400 µg/ml G418 (Gibco). Resistant colonies were counted at Days 11–14. For injection in nude mice G418 resistant clones from the six plates derived from one parental plate were trypsinized, pooled, and injected in two nude mice as described below. In experiments to test the transfected cell population for morphologically transformed foci, no trypsinization was carried out. Transformed foci were scored and isolated 21 days after transfection.

Tests for Tumorigenicity

Cells [XP44RO(Mel) or transformed NIH3T3 cells] were injected s.c. in athymic "nude" mice ($2-3 \times 10^6$ cells/mouse). Tumors appearing after about 20 days at the site of injection were examined histologically and immunochemically [in the case of XP44RO(Mel)-derived tumors]. Tumors originating from NIH3T3 transformants were characterized by Southern hybridization and *in vitro* cultivation of tumor cells in medium containing G418.

Growth in Soft Agar

The procedure used for growing the XP44RO(Mel) cells in soft agar to test for anchorage-independent growth was essentially as described (12). 10^4 cells were plated in growth medium containing 0.37% agar on top of a base layer of 0.5% agar in 6-cm Petri dishes. Colonies comprising more than 10 cells were scored after 14 days.

Cytogenetic Analysis and Immunocytochemical Staining Procedures

Cytogenetic studies were performed on logarithmically growing XP44RO(Mel) cells. The GTG (G-bands by trypsin Giemsa) and RFA (R-bands by fluorescence and acridine orange) techniques were used according to ISCN 1985 (13).

Immunocytochemical staining of S100 protein and a melanoma-associated cell surface antigen (14) was performed on paraffin sections of tumors, and on coverslips cultures fixed in 4% buffered formaldehyde (for S100 protein) or in cold methanol (for the cell surface antigen). Primary antibodies were a polyclonal rabbit antiserum against S100 (obtained from Dakopatts, Copenhagen) and a monoclonal mouse antibody NKI/C3 (purchased from Sanbio, Uden, The Netherlands). As secondary antibodies swine anti-rabbit Ig and rabbit anti-mouse Ig conjugated to horseradish peroxidase (both from Dakopatts, Copenhagen) were used or goat anti-rabbit Ig and rabbit anti-mouse Ig conjugated to fluorescein isothiocyanate (from Supertechs, Bethesda, and Nordic, Tilburg, The Netherlands, respectively).

DNA Procedures

Isolation of genomic, high molecular weight DNA and plasmid DNA, restriction enzyme digestion, gel-electrophoresis, *in vitro* ^{32}P -labeling of DNA probes by nick-translation or the random priming method (15) and Southern blot hybridization were done according to standard procedures as described by Maniatis *et al.* (16). For detection of human *ras* genes in DNA of NIH3T3 transformants appropriate insert fragments of the following plasmids were used: plasmid p6a1 containing a 580-base pair *Sal*/*Eco*RI insert of a human *N-ras* cDNA (17), plasmid

p640 harboring a 640-base pair *Eco*/*Hind*III fragment of the human *Ki-ras* gene (18). The *Ha-ras* gene was visualized using an *Sst* fragment of 2.9 kilobases from the cloned human *Ha-ras* oncogene in plasmid pT24 (19). The dot blot screening procedure for identification of point mutations in activated *ras* oncogenes using synthetic oligonucleotides and *in vitro* DNA amplification has been described in detail previously (20).

RESULTS

Establishment and Phenotypical Characterization of the XP44RO(Mel) Cell Line. From the excised melanoma two cell lines could be established *in vitro*, using the procedure detailed in "Materials and Methods": a tumor cell line designated XP44RO(Mel) and a primary fibroblast strain XP44RO. The tumor cell line was cloned prior to use in the experiments described below. It exhibited a low cloning efficiency (<2%), that could be improved upon cultivation in the presence of a feeder layer of lethally irradiated primary human fibroblasts.

In Fig. 1 a phase contrast photomicrograph of the XP44RO(Mel) cell line is presented. The cells grow in a disorderly array and show prominent dendritic projections, characteristic of melanocytes. When viewed in normal incident light, a minority of the cells exhibits brown pigmentation, as expected for a melanoma.

The tumorigenic potential of XP44RO(Mel) cells was tested in two ways: colony formation in soft agar and injection in "nude" mice. XP44RO(Mel) cells were able to form colonies in soft agar medium albeit with a low efficiency: 2% of the cells inoculated gave rise to a colony. This low, but significant level is probably due to the poor cloning efficiency of this line.

Subcutaneous injection of 2×10^6 cells into athymic "nude" mice yielded slowly growing tumors (three tumors in three mice injected).

Histological examination of these XP44RO(Mel) tumors showed that the degree of pigmentation was lower than in the original tumor, but otherwise the microscopic appearance of the tumor cells was unchanged.

Immunocytochemical staining of tumor sections with antibodies specific for S100 protein and for a melanoma-associated cell surface antigen (NKI/C3) revealed the presence of both of these melanoma markers in the original testis tumor (Fig. 2) and in XP44RO(Mel) induced tumors in the nude mice. Upon staining of XP44RO(Mel) cultures S100 protein was detected in a minority (approximately 5%) of the cells (Fig. 3). All cells in the culture carried the melanoma-associated cell surface antigen (Fig. 4).



Fig. 1. Phase contrast photomicrograph of XP44RO(Mel) cells grown *in vitro*.

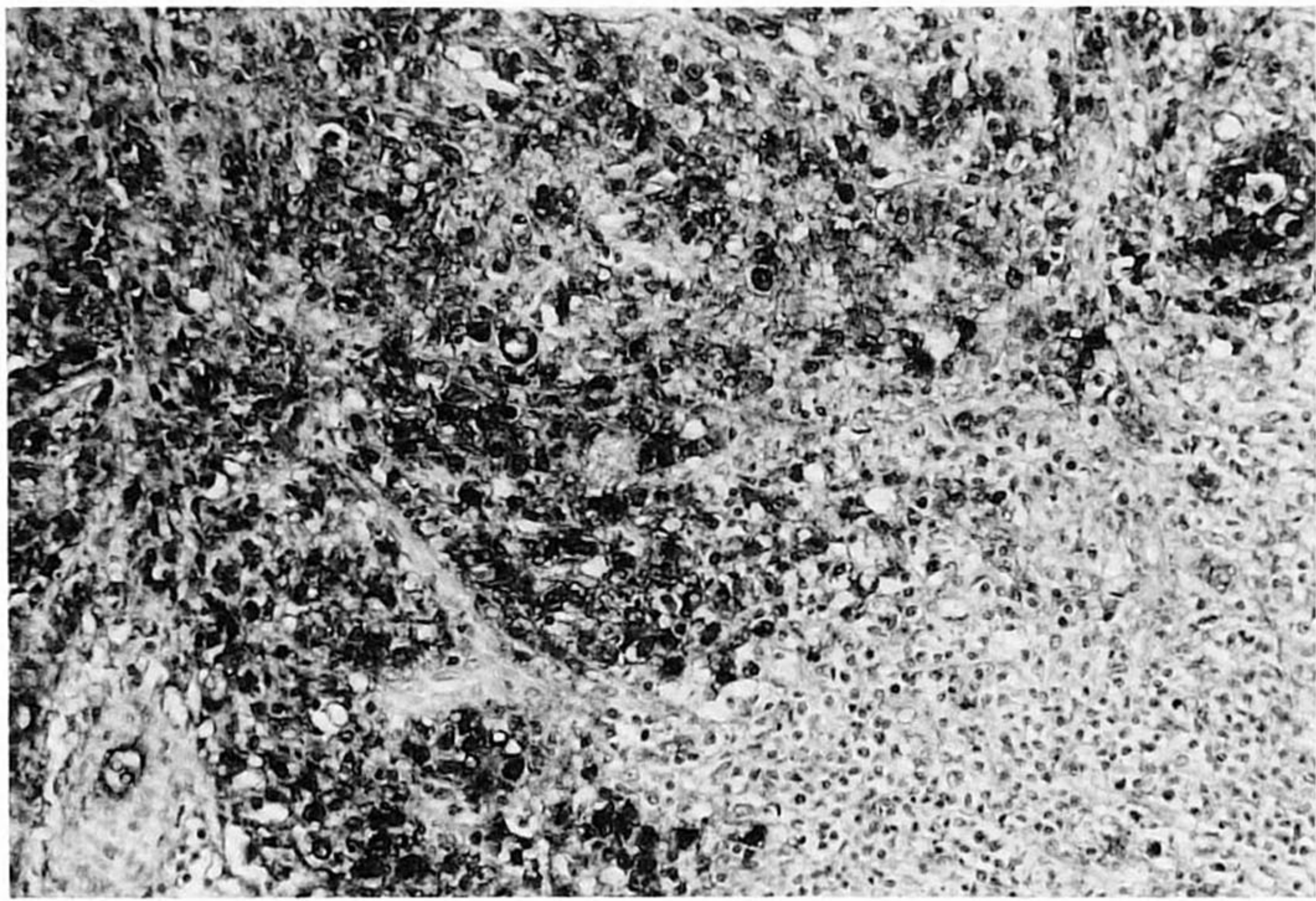


Fig. 2. Immunoperoxidase localization of S100 protein in the original testicular tumor. The melanoma tissue reacts positively with the antiserum, whereas the adjacent nontumorous tissue (*lower right*) is negative.

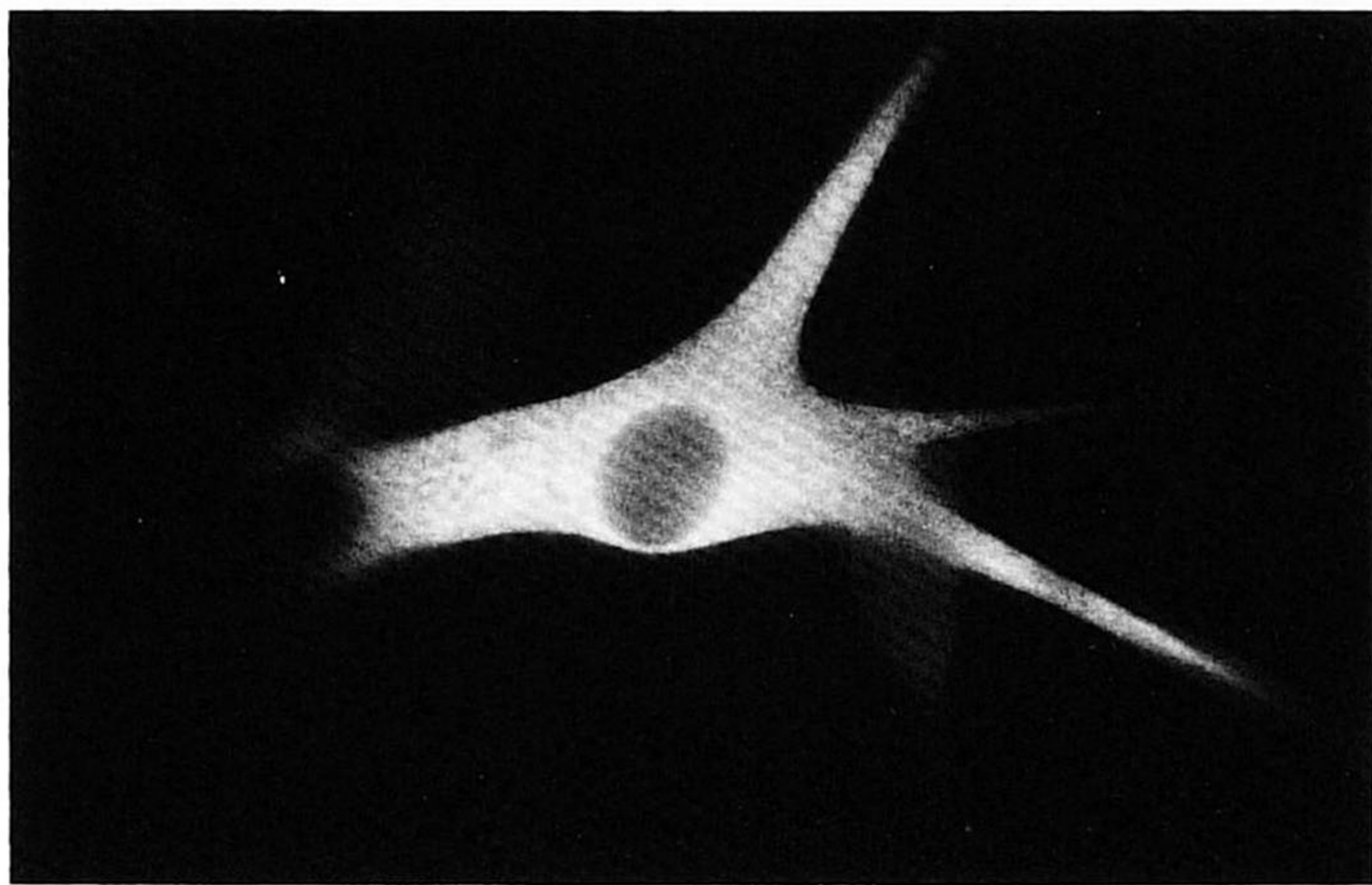


Fig. 3. Immunofluorescence staining of S100 protein in XP44RO(Mel) cells in culture. Besides the strongly stained cell three cells exhibiting only a very weak background fluorescence can be discerned.



Fig. 4. Immunofluorescence staining of melanoma-associated cell surface antigen on XP44RO(Mel) cells in culture. All cells exhibit surface membrane fluorescence.

Cytogenetic Studies. The XP44RO(Mel) line was characterized with respect to its chromosome composition. A representative RFA-banded karyotype is shown in Fig. 5. Cytogenetic analysis revealed 50–53 chromosomes/cell (25 metaphases analyzed), the presence of a number of chromosome abnormalities including one extra X chromosome, trisomies for chromosomes 7 and 20, the presence of an isochromosome 1q, chromosome

aberrations involving chromosome 6, 11, and 22 and the presence of a variable number (1–3) of small unidentifiable marker chromosomes. The karyotype may be described as 50–53,XXY, +i(1q),-6, + marker (6p::?21q). +7, 11p+(6pter→p2.11::11p15→qter), del (11) (q13.3q24), +20, 22p+ (22q::?16q), + one to three small undefined chromosome fragments.

Unfortunately, the karyotype of the tumor material itself could not be determined. Hence, it is not possible to verify whether all these aberrations were also present in the original tumor.

Repair Characteristics. To ascertain that the XP44RO(Mel) cell line isolated had retained its repair-deficient phenotype, colony-forming ability, and level of UDS were determined after UV exposure. Since the primary fibroblast line from a sister of XP44RO (XP5RO) had been assigned by cell fusion to complementation group C in earlier experiments,⁴ XP44RO should also belong to this group. Therefore, XP-C cells were taken as control in these experiments.

As shown by the survival curves in Fig. 6 XP44RO(Mel) cells display a strong sensitivity towards UV irradiation, characteristic of XP cells when compared to a melanoma cell line from a repair competent individual. The UV survival of XP44RO(Mel) is in the same range as that of a SV40-transformed fibroblast line derived from a XP-C patient. Also the level of repair synthesis after a UV dose sufficient to elicit the maximal level of UDS in repair-proficient fibroblasts, is severely reduced in XP44RO(Mel) (25% of wild type) and is not different from that of the reference XP-C primary fibroblast line XP21RO (24%). In earlier experiments a residual excision repair level was found ranging from 25 to 31% in fibroblasts from three sisters of patient XP44RO (XP5RO, XP6RO, and XP7RO). From these data we conclude that the melanoma cell line still displays the XP phenotype.

Assay for Activated Oncogenes in XP44RO(Mel). To examine the oncogenic properties of XP44RO(Mel) high molecular weight genomic DNA was transfected to mouse NIH3T3 cells in coprecipitation with plasmid pMCS (9), carrying the *agpt* gene encoding neomycin resistance. After selection for the uptake and expression of the coprecipitated dominant marker gene (11–14 days after transfection), neomycin-resistant NIH3T3 transformants were scored either for morphological transformation (visible after 21 days) or for tumorigenic potential by s.c. injection of pooled transformants into nude mice.

Foci with a characteristically transformed phenotype appeared with an average frequency of eight per dish (four dishes transfected). When cells were trypsinized and split one in six prior to the start of the neomycin selection (48 h after addition of the DNA precipitate), on the average three transformed foci were obtained per dish. The values are significantly above the background level of spontaneous transformation. Seven foci (three from the first and four from the second transfection) were isolated and their DNA subjected to Southern blot analysis to probe for the presence of human *ras* oncogenes (see below).

To score for the tumorigenic potential of the NIH3T3 transformants pooled (± 1200) neomycin-resistant transformants of each Petri dish (four dishes were used) were injected into two nude mice. After 23 days all eight treated mice had developed tumors at the site of injection. In control experiments in which NIH3T3 cells were transfected with their own DNA and similarly injected into recipient mice, no tumors were observed. Four XP44RO(Mel)-derived NIH3T3 tumors were excised for extraction of DNA. In addition cell lines were established from

⁴ Unpublished results.

Fig. 5. Karyotype from a representative metaphase spread of XP44RO(Mel) cells. R-bands with acridine orange.

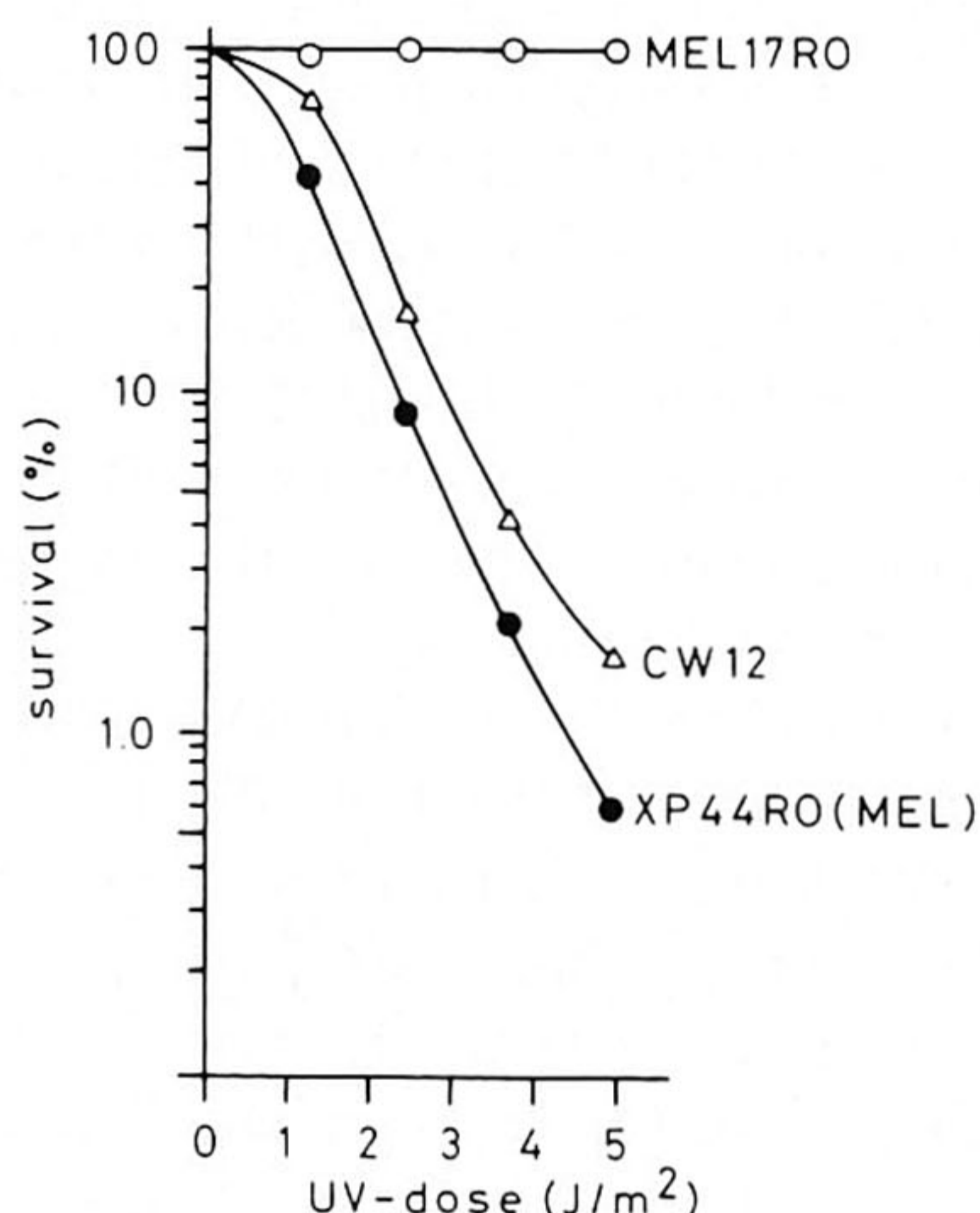
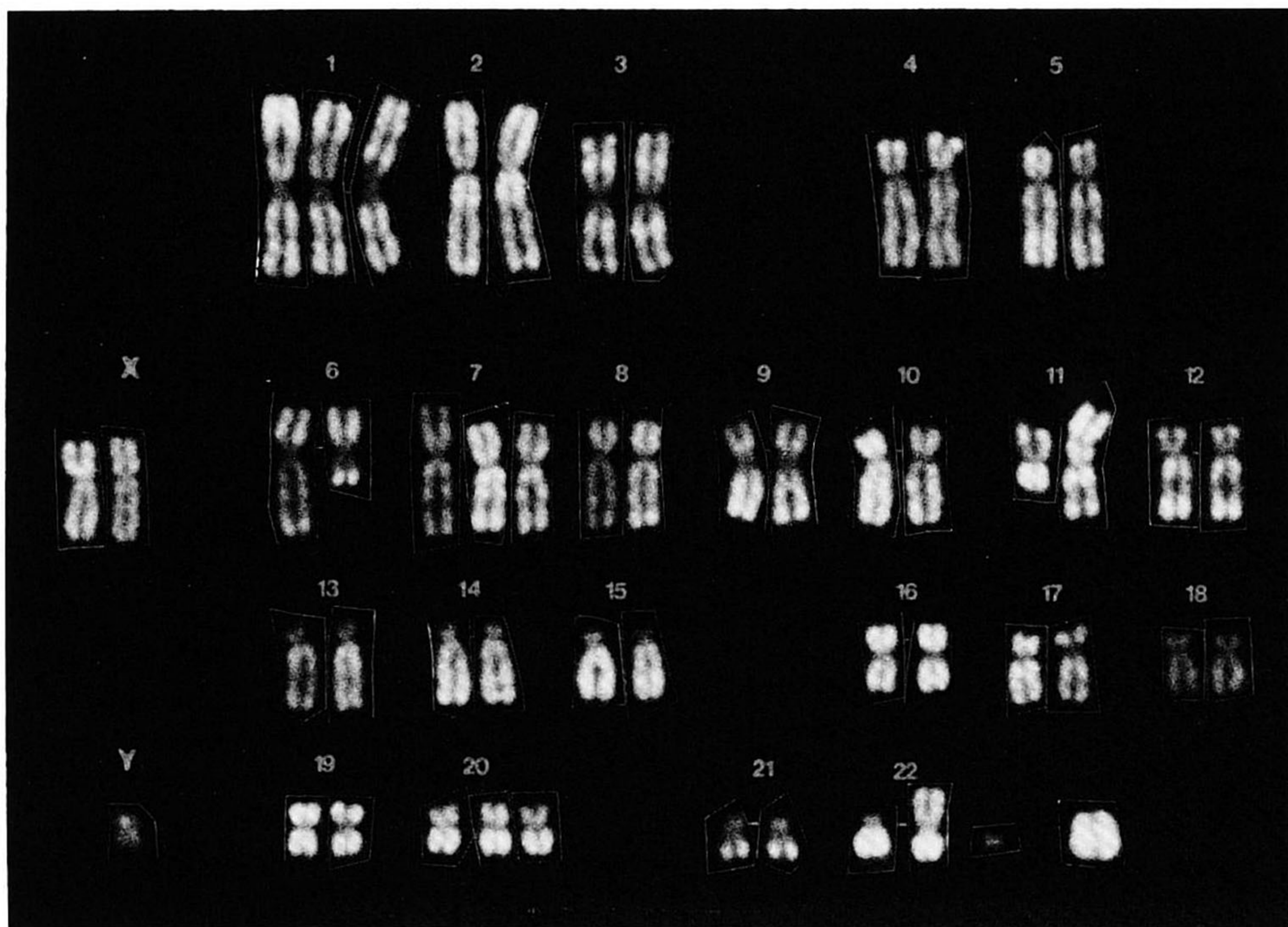


Fig. 6. UV sensitivity of the XP44RO(Mel) cell lines. Survival was examined relative to MEL17RO (a melanoma cell line from a repair proficient individual) and CW12 [an SV40-transformed XP-C cell line (7)].

two tumors. The *in vitro* growing tumor cells displayed a transformed morphology and were resistant against neomycin, indicating that they originated from the transfected NIH3T3 cells.

To determine whether the oncogenic transformation of NIH3T3 was due to the transfer of activated oncogenes present in the genome of XP44RO(Mel), chromosomal DNA of the NIH3T3 transformed foci, mice tumors, and the tumor cell lines derived from the nude mice was digested with restriction endonucleases, size fractionated by gel electrophoresis and after transfer to nitrocellulose hybridized with various ³²P-labeled probes. Hybridization with human cot-1 DNA or a probe specific for the neomycin-resistance gene revealed the presence of considerable amounts of human sequences and multiple copies of the pMCS dominant marker in the genome of all transformants (data not shown). When the filters were probed for the human H- and Ki-ras genes no other fragments than those of the endogenous mouse homologous genes could be detected. [In addition, Southern analysis of DNA of the XP44RO(Mel) tumor cell line did not reveal abnormalities (*i.e.*, amplification

or rearrangements) in the *c-myc* gene]. However, as shown in Fig. 7, when a probe of the human *N-ras* gene was applied strongly hybridizing bands corresponding in size with those of the human genes were visualized in the DNA of all transformants and tumors.

Apparently, during growth in mice amplification of the exogenous *N-ras* gene has occurred, as the signal of hybridizing bands in the tumor DNA is significantly stronger than that in the XP1BR control (one copy per haploid genome) and most of the NIH3T3 foci. These data indicate that the *N-ras* gene in XP44RO(Mel) is responsible for the neoplastic transformation of NIH3T3 cells.

Identification of the Point Mutation in the *N-ras* Gene of XP44RO(Mel). Activation of *ras* genes in human tumors has been found to be the result of point mutations at specific positions in the coding sequence. The mutations have been assigned exclusively to amino acid residues 12, 13, and 61 (see Ref. 21 for a review on *ras* genes). To identify the specific point mutation in *N-ras* of XP44RO(Mel), DNA of this cell line and the original tumor were analyzed by the dot blot oligonucleotide screening procedure (20). This method is based on selective hybridization of a set of synthetic oligonucleotides complementary to all possible mutations in the critical regions of *ras* oncogenes. After amplification of the target sequence by the polymerase chain reaction (22), specific hybridization was found only with the mutation specific 20-mer for position 3 of codon 61 (Fig. 8). The codon CAA in the normal *N-ras* protooncogene has changed into CAC or CAT. This results in the substitution of a glutamine into a histidine residue in the activated *N-ras* protein.

DISCUSSION

The XP44RO(Mel) cell line, described in this paper, represents, to our knowledge, the first cell line derived from a tumor of an XP patient. In the past many attempts have been made by us and others to establish cell lines from XP tumors. However, all efforts have failed. The reason for the success with this specimen is unknown. It may be related to the fact that it was derived from a metastatic melanoma. However, other factors

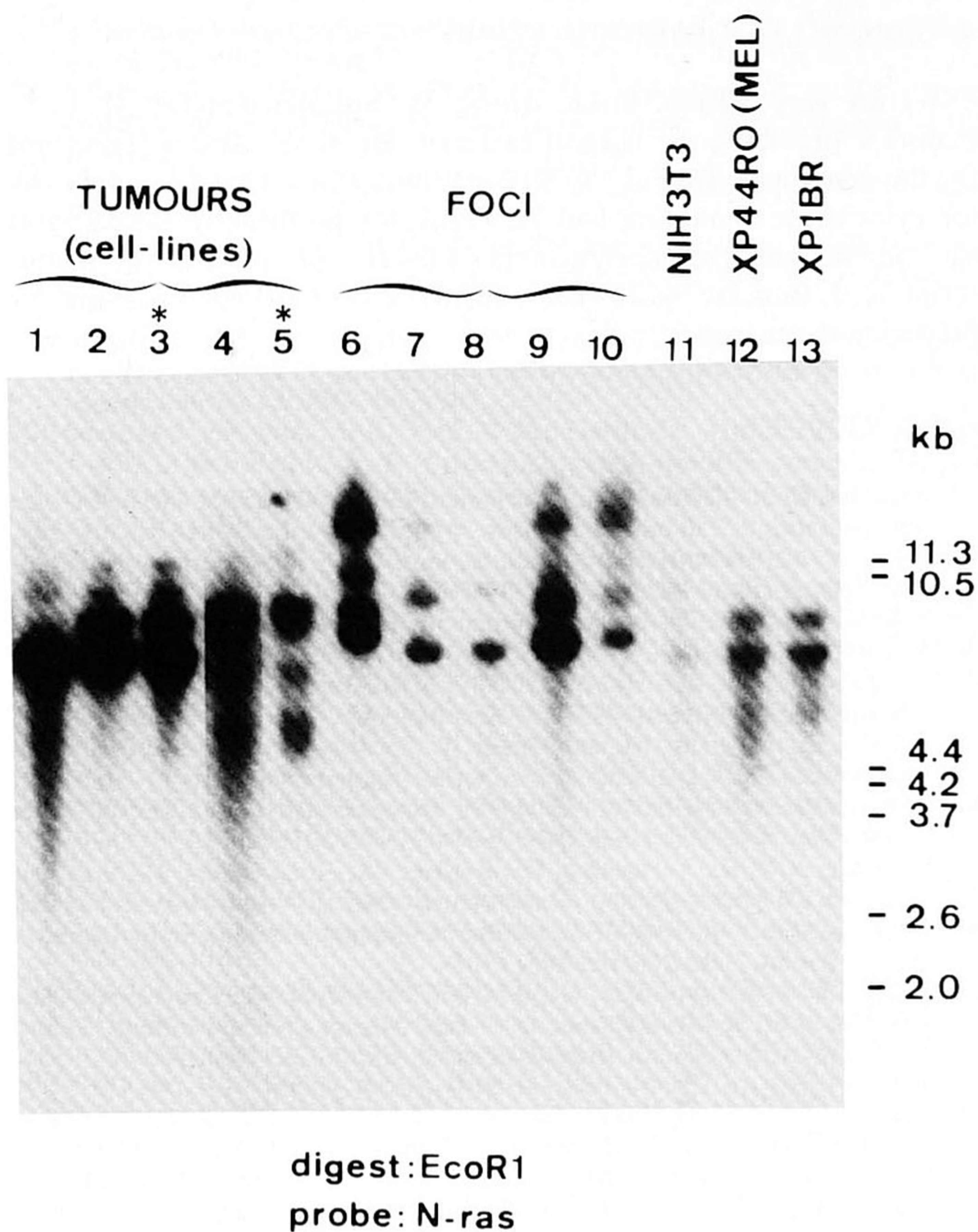


Fig. 7. Southern blot hybridization of DNAs from NIH3T3 transformants or tumors probed for the presence of the human *N-ras* gene. Equal amounts of genomic DNA (10 μ g/lane) was digested with restriction endonuclease *EcoRI*, size fractionated on 0.7% agarose and transferred to nitrocellulose. The filter was hybridized with the ³²P-labeled, isolated 580-base pair insert of plasmid p6al (17), containing a partial cDNA clone of the human *N-ras* gene. Lanes 1, 2, and 4, DNA of tumors appearing in nude mice after injection of pooled G418 resistant NIH3T3 transformants, transfected with XP44RO(Mel) DNA in coprecipitate with plasmid pMCS (9). Lanes 3* and 5*, DNA of the cell lines established from the tumors in lanes 2 and 4, respectively. Lanes 6-10, DNA of different NIH3T3 foci obtained after transfection of XP44RO(Mel) genomic DNA. Lanes 11-13, DNA of NIH3T3 (nontransfected), XP44RO(Mel), and XP1BR (an XP-D cell line with one *N-ras* gene copy per haploid genome) as controls. The high molecular weight bands in lanes 6, 9, and 10 are due to incomplete digestion of the restriction endonuclease.

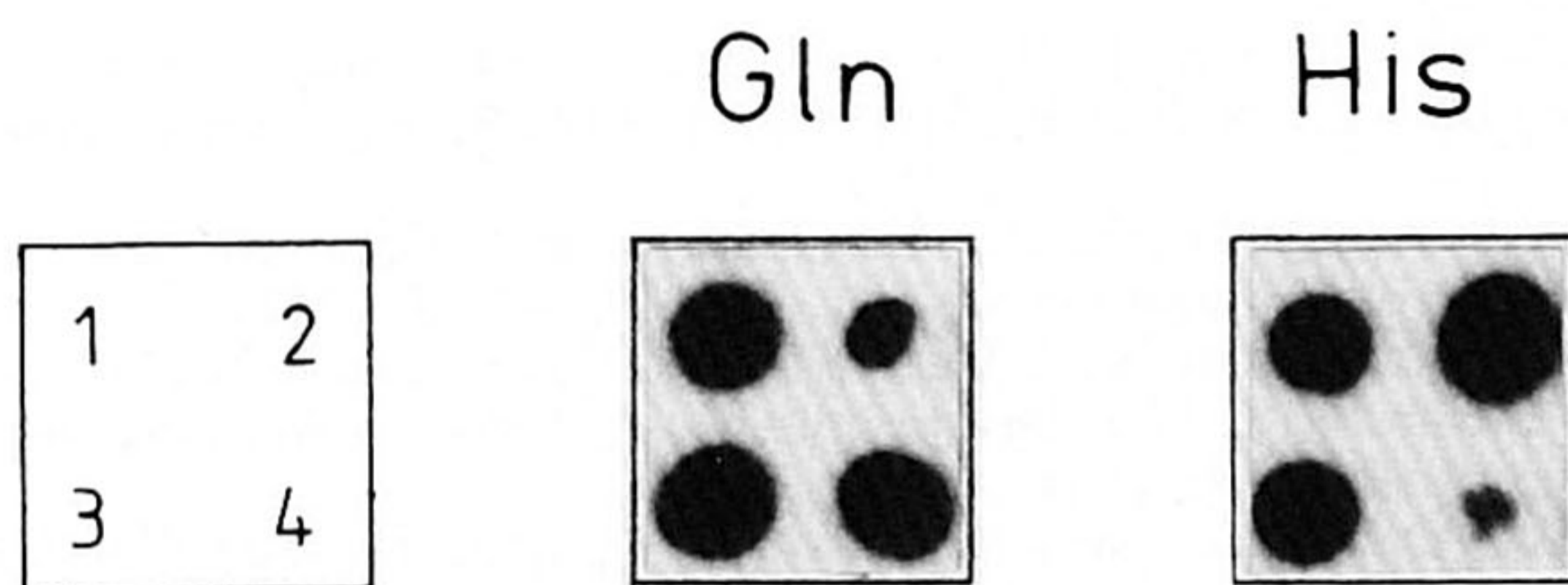


Fig. 8. Dot blot hybridization of ³²P-labeled oligomers specific for the *N-ras* wild-type codon 61 (*gln*) and for the *N-ras* gene mutated at the 3rd position of codon 61 (*His*) to *in vitro*-amplified DNA of the XP44 tumor (1), a NIH3T3 focus obtained after transfection with XP44RO(Mel) DNA (2), the XP44RO(Mel) cell line (3) and a normal cell (4).

or properties of the tumor could have been of crucial importance as well.

Characterization of the XP44RO(Mel) cell line revealed that it had retained its XP features: increased sensitivity to UV and reduced levels of UV induced UDS. From complementation analysis performed on primary fibroblasts of a sister of the patient it follows that XP44RO(Mel) belongs to complementation group C. The cell line exhibited characteristics of a melanoma in accordance with the phenotype of the tumor from

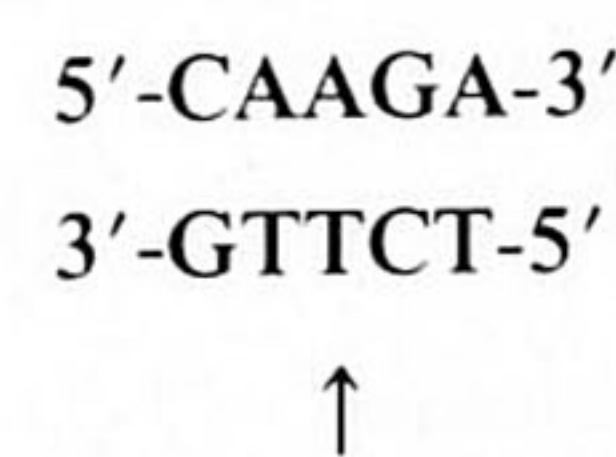
which it was derived. Furthermore, it possessed transformed and tumorigenic properties.

Cytogenetic analysis showed a hyperdiploid karyotype with tetrasomy of 1q, trisomy of 6 p, no. 7, no. 20, and possibly of 21q and 16q, as well as an extra X-chromosome and (partial) monosomy of 6q and 11q. Previous cytogenetic studies reported on malignant melanomas and melanoma cell lines have emphasized the nonrandom occurrence of abnormalities of chromosome 1, particularly 1q, 6, in particular del.6q and of chromosome 7, as well as the frequent occurrence of whole arm rearrangements with breakpoints at or near the centromere of the chromosomes involved (23-28). In the XP44RO(Mel) cell line we observed whole arm rearrangements in three markers, *i.e.*, i(1q), 6q-, and the 22p+. The occurrence of cytogenetic abnormalities involving chromosome 1, notably trisomy for all or a portion of the long arm have been frequently reported in many cancers of hematopoietic as well as nonhematopoietic origin (29, 30) including cases of malignant melanoma (23-25).

Deletion of 6q has been claimed to be specific for malignant melanoma (25-27) and is seen also in this cell line. Numerical abnormalities of no. 7 are frequent in solid tumors and have been implicated in metastasis of various tumors including melanoma (31-34). The alterations registered in chromosomes 11, 16, 20, and 22 might be the result of secondary structural changes as they are more often seen in cultured melanoma cells than in karyotypes established from direct preparation (see Ref. 24). In conclusion the cytogenetic abnormalities registered in XP44RO(Mel) metaphases fit into the general picture emerging from the analysis of other melanomas.

The tumorigenic potential of XP44RO(Mel) cells was apparent from their ability to form colonies in soft agar and to induce tumors in nude mice. Moreover, upon transfection DNA of XP44RO(Mel) transformed NIH3T3 cells and conferred tumorigenicity to these cells, indicating the presence of a dominantly acting oncogene in the genome. Southern blot hybridization revealed that one of the *N-ras* alleles of the tumor cells was responsible for this behavior. Members of the *ras* family (*c-Ha-ras*, *c-Ki-ras*, *N-ras*) are found to be frequently activated by point mutations preferentially at codon positions 12, 13, and 61 in a variety of human tumors and premalignant tissues (21, 35, 36). The fraction of tumors positive for *ras* genes harboring one of the critical base substitutions has increased with improvements of techniques for their detection underlining the importance of this class of oncogenes in neoplastic development; *e.g.*, over one-third of human colorectal cancers (37, 38) and more than one-fourth of acute myeloid leukemias (39) were found to contain mutated *ras* genes. In the latter case a high prevalence of *N-ras* activation was noted. Altered *N-ras* genes have also been demonstrated in several skin tumors including melanomas (40, 41). Finally, it is worth noting that two independent squamous cell carcinomas of one XP patient appeared to harbor-activated *N-ras* genes as well (42).

Hybridization with point mutation specific oligonucleotide probes, performed in this study, indicated that *N-ras* codon 61 was affected at position 3, resulting in a glutamine to histidine amino acid substitution. At the nucleotide level an A·T to C·G, or T·A, transversion had occurred. What type of lesion could have been responsible for this mutation? The nucleotide sequence around the mutated base:



reveals that in the noncoding strand the substituted residue (*arrow*) is situated within a short pyrimidine stretch. This suggests the involvement of UV-induced dipyrimidine photo-products as the initiating DNA injury. Two UV-light photoproducts: cyclobutane dimers and 6-4 photoproducts are most closely associated with mutagenesis. Cyclobutane pyrimidine dimers, the major UV damage, are likely candidates for causing the *N-ras* activation in XP44RO(Mel): both a TT and CT dimer can be formed at the mutated position. The less frequent (by a factor of 10) pyrimidine pyrimidone (6-4) photoproduct, which constitutes one of the major premutagenic lesions at least in *Escherichia coli* (43), is a less likely candidate because this type of damage is not formed at CT dinucleotides and only rarely at TT sites (44). Photoreactivation of UV-irradiated shuttle vectors transiently replicating in repair proficient cells has shown that a large proportion (50–80%) of the mutations scored in this system are associated with or directly derived from cyclobutane dimer lesions (45, 46). In SV40-transformed XP-A fibroblasts the contribution of dimers to the sequence changes observed was even 90% (45). These results demonstrate that at least using shuttle vector target sequences cyclobutane pyrimidine dimers are mutagenic in primate cells.

The UV light-induced mutational spectrum emerging from studies utilizing shuttle plasmids irradiated either before or after transfection to mammalian cells (including XP-A) reveal a predominance of G·C to A·T transitions (45, 47, 48). Most of the transitions occurred at the 3' site of dipyrimidines or could be interpreted as such (45). Also in the codons for the critical amino acids of the *N-ras* gene, there are several possibilities for this type of transition. Interestingly, however, the transversion identified in the *N-ras* gene of XP44RO(Mel) and the original tumor (A·T to C·G or T·A) represents a class of mutations that is either very rare (A·T to T·A) or virtually absent (A·T to C·G) from the spectrum of UV-induced base substitutions observed in wild-type and XP-A cells using shuttle vectors. Very recently, an extensive study was published on the types of mutations induced by UV in the endogenous adenine phosphoribosyltransferase locus of Chinese hamster ovary cells (49). In this survey also G·C to A·T transitions were found to predominate (70%) whereas A·T to T·A or C·G transversions were noted in approximately 15% of the single base substitutions. However, it should be kept in mind, that the studies mentioned above deal with mutations causing gene inactivation, whereas this paper concerns a highly selected gene activation event, which is only possible in a restricted set of codons. The mutational spectrum of these two types of DNA alterations may not always correlate. Analysis of additional mutations in XP patients and normal individuals will have to reveal whether consistent differences exist between *in vivo* occurring nucleotide changes in endogenous genes and *in vitro* generated mutations in cultured cells. In this respect it is worth noting that very recently two independent squamous cell carcinomas of one XP patient were found to harbor also *N-ras* genes activated at codon 61 by an A·T to T·A or C·G transversion.⁵ Furthermore, activation of *N-ras* predominantly at codon 61 was also found after UV irradiation of the normal gene and subsequent transfection to NIH3T3 cells (50). These findings stress the significance of the observations reported here.

The XP44RO(Mel) cell line described here should prove to be useful for extending the insight into the molecular basis and consequences of the repair defect in XP.

⁵ A. Sarasin, personal communication.

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