Loss of Heterozygosity at Chromosome 1q22 in Basal Cell Carcinomas and Exclusion of the Basal Cell Nevus Syndrome Gene from This Site

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ABSTRACT

Basal cell carcinomas, the most common human tumors, generally appear sporadically and in small numbers. Rarely, they may appear in great numbers and at an earlier age as a manifestation of the basal cell nevus syndrome, an autosomal dominant inherited disorder. Drawing on the retinoblastoma paradigm, we have begun a search for tumor suppressor genes important in the development of basal cell carcinomas by comparing DNA of tumors and normal cells. Loss of heterozygosity, a frequent marker of the site of tumor suppressor genes, was found at chromosome 1q in one-third of the tumors studied. However, comparison of the inheritance of DNA markers versus the inheritance of the basal cell nevus syndrome in one large kindred excluded this area of chromosome 1q as the site of the gene whose abnormality causes this hereditary disease. These data suggest that large deletions may accompany the development of cutaneous, low-grade tumors just as they accompany the development of visceral, high-grade cancers.

INTRODUCTION

During the past 15 years, it has become clear that genetic changes underlie the conversion of cells from the normal to the malignant state. The best-studied such alterations have been mutations causing activation or amplification of dominantly acting oncogenes—genes whose products drive the cell to excess proliferation, invasion, etc. More recently, the existence of a second class of genes important in carcinogenesis has become obvious. These are the tumor suppressor genes, whose normal products slow cellular proliferation and/or enhance cellular differentiation. Unlike the first type of oncogene, it is the loss of function of both alleles of these genes, not the altered function of one allele, that causes the cell to become malignant.

The prototype of such a tumor suppressor gene is the retinoblastoma gene (2). Loss of functional retinoblastoma gene product is a crucial step in the development of ocular cancer and indeed may be the necessary and sufficient requirement. Mutation of both copies of the gene may occur somatically, or one defective allele may be inherited, in which instance only a single mutation need occur somatically (3). Disease in the hereditary form is inherited as an autosomal dominant trait and is characterized clinically by earlier age of onset and by the frequent occurrence of multiple independent tumors.

One common mechanism for loss of a tumor suppressor gene is the physical deletion of a large part of a chromosome on which such a gene resides (4). Such large scale deletion provides a large "target" for molecular biological analysis, e.g., by comparison of restriction fragment length polymorphisms in DNA from the patient's normal and tumor cells. If the normal DNA is heterozygous for such a marker, deletion of one allele converts the pattern of heterozygosity to one of hemizygosity (or, if there is duplication of the retained segment, to homozygosity). Such loss of heterozygosity in tumor cells suggests that a tumor suppressor gene lies in the deleted segment, and such loss of heterozygosity has pointed the way to cloning of tumor suppressor genes on chromosomes 11p (5, 6), 17p (7), and 18q (8), as well to cloning of the retinoblastoma gene (9-11).

BCCs are by far the most common human cancer, occurring in some populations as commonly as all other cancers combined. Like patients with sporadic retinoblastomas, patients with sporadic BCCs generally develop only one (or a few) BCCs and do so at a relatively advanced age. As with retinoblastomas, there also are kindreds in which patients develop multiple BCCs at an early age, and this trait is inherited as an autosomal dominant disease—the BCNS (McKusick no. 10940). In addition to developing multiple, early onset BCCs, patients with BCNS also have palmar and plantar pits, keratoacanthomas of the jaw, skeletal abnormalities, and various extracutaneous tumors (12).

Drawing on the retinoblastoma model, we have begun an effort to find the gene whose abnormality leads to the BCNS by searching for loss of heterozygosity in BCCs in the hope that regions involved in loss of heterozygosity would identify candidate regions for linkage analysis and would indicate regions in which a tumor suppressor gene important in BCC development might be found. To start, we have examined BCC DNA for loss of heterozygosity in regions in which such loss has been reported in other tumors and have performed linkage analysis in a large kindred with BCNS in regions of loci that might be considered candidate genes for this disease. Specifically, weak linkage has been reported with chromosome 1q markers (13), and one family has been reported in which there is apparent co-segregation of BCNS and Charcot-Marie-Tooth disease (14), autosomal forms of which have been linked to chromosomes 1q (15), 17p (16-18), and 19q (19). Hence, we have performed linkage analysis in these 3 regions.

MATERIALS AND METHODS

Patient Samples. Cutaneous lesions with the clinical appearance of BCCs were removed by curettage rather than by sharp dissection so as to minimize the admixture of normal cells. Sclerotic BCCs were not used. If not previously confirmed histologically, a portion of the tumor was taken for histopathological examination, and the remainder was frozen. Blood samples were anticoagulated with EDTA and stored frozen in plastic tubes. DNA was extracted from blood by standard techniques and from tumors as follows: frozen tumors were minced on ice; incubated at 4°C for 20 min in 320 mM sucrose, 5 mM MgCl₂, 1% (v/v) Triton X-100, 10 mM Tris, pH 7.5; and then centrifuged at 2500 × g for 10 min. The pellet was suspended in 4.25 mL 75 mM NaCl, 25 mM EDTA, pH 8.0, containing 0.5% sodium dodecyl sulfate and

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proteinase K, 200 µg/ml, and incubated with gentle shaking for 18 h at 55°C. If solubilization was visibly incomplete, more sodium dodecyl sulfate, NaCl:EDTA solution was added, and the incubation was continued for another 2-6 h. The solution was extracted with an equal volume of phenol, of phenol:chloroform:isoamyl alcohol (25:24:1), and of chloroform:isoamyl alcohol (24:1). The aqueous phase was transferred to a fresh tube, and 0.1 volume of 3 M sodium acetate, pH 7.0, and 2 volumes of ethanol were added. After inversion to mix, DNA was removed with a sterile needle. Higher yields of DNA were obtained if the aqueous phase was centrifuged after incubation for 18 h at -20°C, and the DNA was of lower molecular weight and was considerably contaminated with RNA. Hence, it was used only with tumors of 2-4-mm diameter, from which only 50 µg of DNA could be recovered. Larger tumors, however, often yielded more than 1 mg of DNA. Yields of DNA were not enhanced by pretreatment of minced tumors with collagenase and/or trypsin-EDTA.

This study was approved by the University of California San Francisco Committee on Human Research, and informed consent was obtained from all subjects prior to their participation.

DNA Analysis. DNA was digested with restriction endonucleases according to the manufacturers' instructions, separated by electrophoresis in agarose, and transferred to sheets of Hybond-N (Amersham Corp.) or Immobilon-N (Millipore Corp.). Loci studied (probe/restriction enzyme used) were as follows: D1S57 (pYNZ22/MspI), NGFB (N8C6/RflI), D1S90 (RL3/MspI), MUC1 (pMUC10/Hinfl), SPTA1 (3021E/MspI), FCG2 (FCGII cDNA/TagI), D1S61 (pMLAJ1/Hinfl), D1S66 (pHBI40/MspI), AT3 (pAT3c/FlI), D1S65 (pEKH7.4/TagI), HRAS (pUC EJ 6.6/ BamHI), D17S5 (pYNZ22.1/MspI), D17S71 (pA10-41/MspI), D19S58 (pL17.1/MspI), and APOC2 (pCII-711/TagI). Probes were generous gifts of B. Forget (3021E1), S. Gendler (pMUC10), K. Moore (FCGII cDNA), Y. Nakamura (pYNZ22, pHBI40, and pEKH7.4), and R. White (pMLAJ1) or were obtained from The American Type Culture Collection, Rockville, MD. In each instance, the band patterns corresponded to those described in the literature (20, 21). Linkage analysis was performed with LIPED.

RESULTS

Loss of Heterozygosity in Sporadic Basal Cell Carcinomas. Despite numerous attempts, we found that we could not extract enough DNA from the more commonly seen smaller (e.g., 3–4-mm) BCCs to allow multiple analyses of a single tumor. In contrast, extraction of larger (e.g., 8–10 mm) BCCs produced DNA ample for multiple analyses. Since the patients with BCNS visited their dermatologists frequently for removal of new lesions, larger BCCs were available from BCNS patients only rarely and more commonly were available from patients with sporadic, comparatively neglected tumors. All of these tumors were from sun-exposed areas of Caucasian individuals, and the ages of the patients at the time of removal were 38–81 years.

We analyzed 22 BCCs (sporadic, 19 tumors from 18 patients; BCNS, 3 tumors from 3 patients) for loss of heterozygosity either at chromosome 1q or at several other regions in which loss of heterozygosity has been reported frequently for extra-cutaneous cancers (Table 1; Fig. 1). Of the regions surveyed, chromosome 1q22 had the highest frequency of loss of heterozygosity at 33%. Analysis of tumors with chromosome 1q deletions using multiple probes from this area indicated the shortest overlapping region of deletion between the genes for peanut urinary mucin (MUC1) and for FCG2 (Fig. 2), a distance estimated at approximately 20 centimorgans (22). Using probes identifying the 5 polymorphic sites, we detected loss of heterozygosity only at chromosome 1p in each of 2 tumors from BCNS patients and at no site in the tumor from the third BCNS patient. We observed no preferential loss of larger or smaller bands.

Linkage Analysis in BCNS. Blood samples were obtained from 53 members of a large kindred (M1) with BCNS (Fig. 3). A portion of this kindred, which is unusual in that both BCNS and inflammatory bowel disease are present (inherited apparently independently), has been the subject of a previous clinical report (23). All members diagnosed as affected with BCNS had at least 2 of the following: (a) at least one BCC prior to the age of 20 or multiple BCCs thereafter; (b) multiple palmar and/or plantar pits; and/or (c) documented jaw cysts. No affected member failed to develop one of these signs by age 18 (usually

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<td>Chromosome location</td>
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<td>Loss of heterozygosity</td>
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<td>1p           1q22   1q32   11p15  17p13.3</td>
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<td>4/21  7/21    2/16   1/12   2/11</td>
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* Loci assessed were 1p-D1S57 and NGFB, 1q22-MUC1, 1q32-D1S65, 11p15-HRAS, and 17p13.3-D17S55.
common adult cancers, in which tumorigenesis is accompanied by multiple genetic steps. Thus, in colon cancers, perhaps the tumor best studied for sequential mutations, loss of heterozygosity occurs at all chromosome arms, albeit at varying frequencies, with an average of 4 to 5 deletions per tumor (25). Approximately 25% of colon tumors have loss of heterozygosity of chromosome 1q, a frequency intermediate among chromosome arms in colon cancers (25). Fearon and Vogelstein (26) have suggested 2 explanations for their finding of loss of heterozygosity at many sites: (a) each of these sites indeed harbors a tumor suppressor gene; or (b) much of the loss is neutral with regard to tumor growth but occurs during disorderly mitoses in which regions containing true tumor suppressor genes are lost. We interpret our finding of a relatively high incidence of chromosome 1q loss of heterozygosity with confinement to a relatively short region of overlapping deletion, coupled with a low incidence of loss of heterozygosity at several other sites, as more consistent with the first explanation—mitoses causing loss of heterozygosity at multiple sites seem, at least in this relatively low-grade cancer, to be relatively infrequent.

Chromosome 1q alleles also have been reported to be lost in tumors of another epithelial-derived tissue, the breast, and there is some concordance between the chromosome 1q region lost in BCCs and that lost in breast cancer (27, 28). Recently, chromosome 1q also has been identified as the site of a p53 and DCC, genes whose loss occurs later in development of cancers such as those of the colon (26). However, such correlation requires more study, for we have no evidence as to whether there is normal functioning of transcripts from these genes in BCCs.

The BCCs studied were, by their size, an unusual group, and it is possible that the genetic changes found are not characteristic of the more common, smaller BCCs. Even such larger BCCs metastasize only very rarely, and as a first approximation this correlates with the lack of BCC loss of heterozygosity in the regions of p53 and DCC, genes whose loss occurs later in development of cancers such as those of the colon (26). However, such correlation requires more study, for we have no evidence as to whether there is normal functioning of transcripts from these genes in BCCs.

Others have reported loss of heterozygosity of distal chromosome 11p in BCCs at the c-Ha-ras oncogene—4 of 16 tumors (30)—an incidence a bit greater than we found in this area—1 of 12 tumors. Together, 5 of 27 tumors, this frequency of loss of heterozygosity (19%) still is lower than that which we found at chromosome 1q (33%). This site has been of special interest since the first report of its frequent activating mutations and loss of heterozygosity in experimentally derived skin tumors (31), but ras gene mutations appear to occur infrequently in human BCCs (32, 33). We do not know whether the few BCCs with chromosome 11p loss of heterozygosity have activating point mutations in the remaining alleles.

Fig. 2. Loss of heterozygosity of chromosome 1q markers in 7 (numbered) basal cell carcinomas. Numbers at left, genetic map distance (in centimorgans) from chromosome Ip telomeric-most probe (22, 24, 40). •, Retention of heterozygosity at indicated locus; D, loss of constitutional heterozygosity in tumor at indicated locus; , areas of retained heterozygosity; ---, maximum extent of deleted regions (i.e., of loss of heterozygosity) in tumors.

Fig. 3. Pedigree of BCNS-Mi family. Persons too young to be judged unaffected are not shown. Shaded areas, affected with BCNS.
Linkage Analysis of BCNS. Weak linkage of BCNS to the Duffy blood group on chromosome 1q (13), and one family with co-segregation of BCNS and Charcot-Marie-Tooth disease, one type of which is produced by an abnormality of a gene close to that for FcgRII, has been reported (14). Hence, we were most encouraged to find a relatively high incidence of loss of heterozygosity at that region and then disappointed to find that linkage analyses in our family as well as that published by others in abstract form exclude this area as the site of the BCNS gene (34). This is analogous to the findings in several other inherited tumors, in which sites of linkage of inherited forms of cancer differ from sites of high incidence of loss of heterozygosity, e.g., linkage of MEN2 to chromosome 10q (35, 36) but high loss of heterozygosity of chromosome 1p in phaeochromocytomas (37) and linkage of familial adenomatous polyposis to chromosome 5q (38, 39) but high loss of heterozygosity at chromosomes 17p and 18q in colon cancers (25).

We also searched for linkage to chromosomes 17p and 19q regions to which other forms of Charcot-Marie-Tooth disease have been linked. Linkage was excluded to each of these regions.

In conclusion, this study gives evidence that the mutations underlying the conversion of keratinocytes to the common, locally invasive but non-metastasizing basal cell carcinomas resemble one type of those underlying extracutaneous carcinogenesis in that both frequently have deletions of large chromosomal regions. Careful comparison of the mutations in these 2 classes of tumors may provide clues to help explain clinically crucial differences in their biological behavior.

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REFERENCES

BASAL CELL CARCINOMA: LOSS OF HETEROZYGOSITY


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