

Cytogenetic Analysis of 33 Basal Cell Carcinomas¹

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ABSTRACT

Cytogenetic analysis of short-term cultures from 33 basal cell carcinomas (BCC), a type of neoplasm for which no previous karyological data exist, revealed clonal chromosome aberrations, all of them different, in 8 tumors. In 2 cases, 2 cytogenetically unrelated clones were detected, suggesting a multicellular origin in at least a subset of BCC. A remarkably high level of nonclonal structural rearrangements, mostly in the form of seemingly balanced translocations, was found in 23 tumors; namely, in 6 of 8 BCC with clonal karyotypic abnormalities and in 17 of 25 without. It is possible that some of these aberrations represent additional neoplastic clones, thus indicating an even higher level of cytogenetic heterogeneity in BCC.

We think that the most likely interpretation of the results is that BCC may have a multicellular origin, reflecting field cancerization of the skin. During subsequent tumor development, the selection pressure narrows down the number of clones that infiltrate the surrounding tissue. The finding by karyotypic analysis of some apparently monoclonal, some polyclonal BCC, may reflect that different tumors have been examined at different points in the clonal evolution of the neoplastic cells.

INTRODUCTION

BCC³ is by far the most common type of skin cancer. Because of its ability to infiltrate locally, it is usually considered a malignant tumor, but it hardly ever metastasizes. Several predisposing factors have been identified: light skin color in association with prolonged sun exposure, large doses of X-rays, and genetic disorders such as xeroderma pigmentosum and Gorlin's syndrome (1).

A large body of data now indicates that the acquisition of genetic rearrangements is an important event in carcinogenesis (2). Particularly important for this conclusion have been the results of chromosome analyses of hematopoietic malignancies, in which certain chromosome aberrations are nonrandomly associated with different tumor types (3). Molecular analyses in, for example, chronic myeloid leukemia and Burkitt's lymphoma have provided further insight into how the chromosomal rearrangements through activation of cellular oncogenes can give proliferative advantage to the cells (4, 5). Recent technical improvements also have made solid tumors accessible for cytogenetic analysis, and here too the general conclusions drawn from the study of leukemias and lymphomas seem to hold (2).

Some tumor types, one of them BCC, have hitherto remained cytogenetically uncharacterized. In this paper, we report the karyotypic analysis of short-term cultures from 33 BCCs.

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³ The abbreviations used are: BCC, basal cell carcinoma.

MATERIALS AND METHODS

The material consisted of 33 BCCs from 25 individuals (Table 1). Thirty-one were primary tumors, and 2 (cases 1 and 19) were local recurrences. Eighteen tumors were removed from the face, 6 from the back, and 9 from the extremities. Case 5 was the only patient with known hereditary predisposition to BCC (Gorlin's syndrome). Patients 1 and 24 had received therapeutic radiation to the areas where their tumors were located. The clinical and histopathological characteristics of the 8 cases with tumors with clonal chromosome aberrations were as follows.

Case 1. A 90-year-old woman presented with a 30- x 30-mm large tumor on the back of her head. Radiotherapy was given, but 1 year later she had a local recurrence which was excised. Histopathological examination revealed a BCC of predominantly adenoid type, with some solid features and a pronounced inflammatory reaction.

Case 2. A 61-year-old woman had had multiple BCCs for 20 years, treated with either surgical excision, local 5-fluorouracil, or freezing. Now, 7 skin tumors (5 BCCs and 2 basal cell papillomas) were excised from different parts of her body. The histopathological diagnosis in tumor 2d was BCC of superficial type, with a moderate inflammatory reaction.

Case 3. A 79-year-old woman had noticed a skin lesion near the medial corner of her left eye for 5 months. Histopathological examination of the 7- x 8-mm large tumor revealed a solid, partly cystic BCC with some adenoid features and a moderate inflammatory infiltrate.

Case 4. A 69-year-old man, who had been treated in 1978 with radiotherapy and surgery because of a carcinoma of the larynx, had suffered from multiple facial BCCs for the last 10 years, all of which had been surgically excised. Now, 2 more BCCs were removed from the nose and the chin. The histopathological diagnosis in case 4b was BCC of solid type, with some cystic features and a mild inflammatory reaction.

Case 5. A 76-year-old woman with Gorlin's syndrome had had multiple BCCs for 44 years; all were treated with radiotherapy and surgical excisions. Now, 2 lesions were removed from the chin (chronic inflammation with squamous cell hyperplasia) and the upper lip (BCC). The BCC was of solid adenoid type with a mild inflammatory reaction.

Case 6. A 78-year-old man had noticed a slowly growing tumor on the left side of his nose, close to the upper eye lid. Histopathological examination of the 10- x 10-mm large lesion showed a solid, partly keratinizing BCC and a pronounced inflammatory reaction with fibrosis.

Case 7. A 49-year-old woman had noticed a slowly growing skin lesion on her left hip for 3 years. Histopathological examination of the 10- x 15-mm large tumor revealed a BCC of superficial type with a moderate inflammatory reaction.

Case 8. A 79-year-old woman had noticed 2 slowly growing red lesions on her back for 1 year. Histopathological examination of tumor 8a, 10 x 15 mm large, revealed a BCC of superficial type with a moderate inflammatory reaction.

The fresh tumor samples intended for karyotypic analysis (neighboring parts of all lesions were examined histologically) were minced with scissors and incubated overnight in 0.2% collagenase. The disaggregated cells were plated on glass chamber slides in RPMI 1640 medium supplemented with fetal bovine serum, glutamine, antibiotics, cholera toxin, epidermal growth factor, and insulin (6). Daily microscopic

Table 1 Clinical data and cytogenetic findings in 33 basal cell carcinomas

Case No.	Age (yr)/sex	Tumor site	No. of mitoses/ no. of metaphases with nonclonal structural chromosome rearrangements	Clonal abnormalities
1	90/F	Head	100/26	46,XX,t(8;14)(p23;q21),t(10;19)(q21;q13)=7
2a ^a	61/F	Head	33/3	
2b		Groin	14/0	
2c		Groin	27/2	
2d		Back	45/16	46,XX,t(5;6)(q11;q27)=2
2e		Back	28/6	
3	79/F	Head	21/0	46,XX,t(4;15)(p14;q15),t(5;19)(p13;q13)=3
4a	69/M	Head	27/9	
4b		Head	34/24	46,XY,t(16;?)(p13;?)=6/46,XY,t(1;19)(q23;p12)=2
5	76/F	Head	42/2	45,X,-X,t(4;5)(p16;q33)=20/46,XX,del(9)(q22)=3
6	78/M	Head	25/0	45,X,-Y=7
7	49/F	Hip	23/1	47,XX,+12=2
8a	79/F	Back	20/11	46,XX,t(3;12)(q21;q24)=2
8b		Back	16/5	
9	78/F	Head	12/2	
10	67/M	Head	11/1	
11	47/F	Head	20/0	
12	66/M	Head	25/1	
13	54/M	Head	41/3	
14	95/F	Head	34/15	
15	65/M	Leg	25/2	
16	84/F	Head	25/0	
17a	51/F	Leg	25/0	
17b		Leg	25/4	
18	82/F	Head	23/0	
19	79/M	Head	25/3	
20a	82/M	Leg	11/0	
20b		Leg	51/6	
21	73/M	Back	25/0	
22	81/M	Head	5/0	
23	76/F	Back	49/12	
24	81/F	Axilla	15/13	
25	53/F	Head	25/2	

^a Letters following the numbers indicate that more than one tumor was studied from the same patient.

inspection of the cultures revealed a mixture of epithelial and fibroblast-like cells. The *in situ* harvest after 5–15 days included exposure to colcemid for 12 h, followed by hypotonic treatment in 0.3% NaCl and repeated fixations in methanol/acetic acid. After incubation overnight at 60°C, the preparations were G-banded with Wright's stain.

To be accepted as clonal (7) in the subsequent cytogenetic analysis, the same structural rearrangement or chromosomal gain had to be present in at least 2 metaphases, either from different *in situ* preparations or from well-separated areas on the same primary culture slide. Loss of a chromosome had to be detected in at least 3 metaphases.

RESULTS

Numerical and/or structural clonal chromosome aberrations were detected in 8 of the 33 BCCs (Table 1; Fig. 1). In 2 of them (tumors 4b and 5), 2 cytogenetically unrelated clones were present. Nonclonal structural changes, almost always in the form of apparently balanced translocations, were found in 23 cases (in 6 of 8 tumors with and in 17 of 25 without clonal changes), where they were present in 4–70% of the cells analyzed (Table 1). In 8 tumors, neither clonal nor nonclonal aberrations were detected.

DISCUSSION

The cytogenetic findings in BCCs seem to differ in 3 respects from what has usually been observed in most other solid tumors and in hematological malignancies: there appears to be no common cytogenetic denominator, cytogenetically unrelated clones are present in a substantial proportion, and the tumors contain a high frequency of cells with nonclonal structural changes.

Whereas most cytogenetically analyzed tumor types have nonrandom chromosome aberration patterns (2), all 8 BCCs with clonal changes displayed different abnormalities. Most clones were very small, but we are confident that the clonality criteria applied were stringent enough to exclude the possibility that they originated *in vitro*. The aberrations presented in Table 1 should therefore represent *in vivo* clones. The numerical changes, *i.e.*, +12, -X, and -Y, have previously been associated with several types of neoplasia (8), and loss of both the X and Y chromosomes has also been described in nonneoplastic cells (9, 10). Of the structural rearrangements seen, only del(9)(q22) has been consistently associated with neoplasia (8). Although the series of 8 BCCs is not large and several histopathological subtypes are included, the cytogenetic heterogeneity detected raises the question of whether all, or indeed any, of the aberrations are of pathogenetic importance. If they are important, there obviously must exist a large variety of genetic pathways through which the genesis or progression of BCCs can be accomplished. It might even be that some of the nonclonal aberrations represent additional malignant clones, only that they were too small to be detected in enough cells to fulfill the criteria for clonality, thus indicating an even higher level of cytogenetic heterogeneity in BCCs.

Regardless of whether the clonal chromosome aberrations represent cytogenetic epiphenomena or pathogenetically significant events, the parallel existence of 2 unrelated abnormal clones in 2 of the BCCs indicates that these tumors were polyclonal at the time of analysis. The finding of independent clones is rare in other neoplasms (11), a fact that, together with the results from studies of other tumor markers (12–14), has been interpreted as evidence that neoplasms develop from a

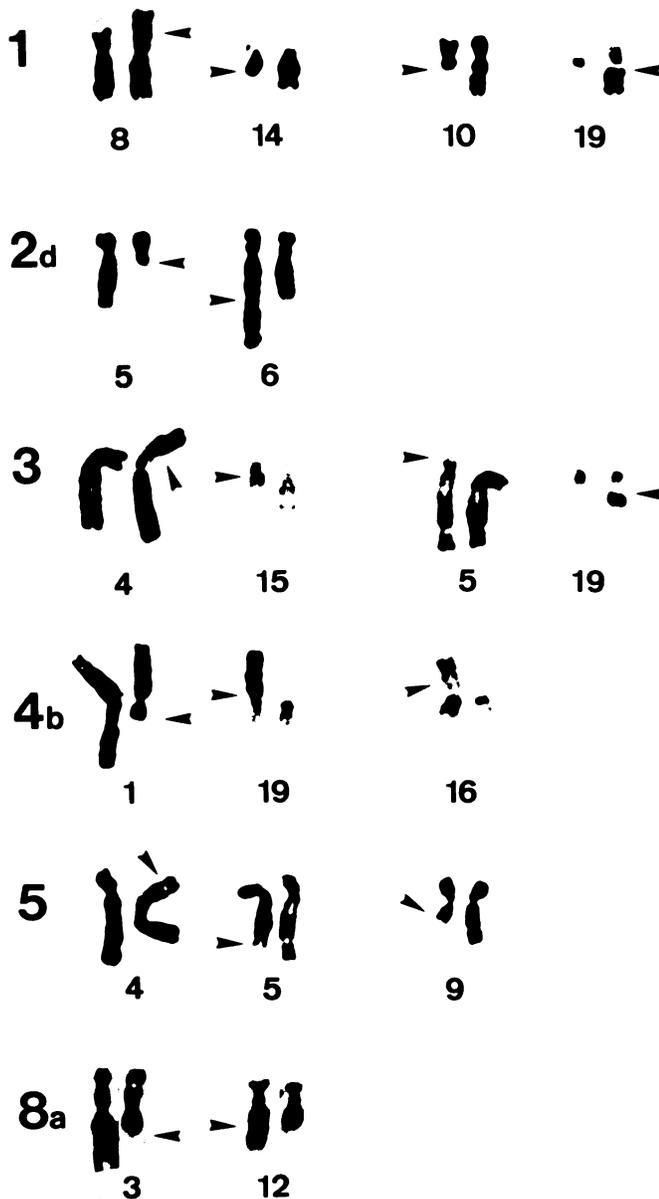


Fig. 1. Partial karyotypes illustrated the different structural chromosome aberrations in 6 of the 8 basal cell carcinomas with clonal changes (tumors 6 and 7 had only numerical changes). Tumor 1, $t(8;14)(p23;q21)$ and $t(10;19)(q21;q13)$; tumor 2d, $t(5;6)(q11;q27)$; tumor 3, $t(4;15)(p14;q15)$ and $t(5;19)(p13;q13)$; tumor 4b, $t(16;?)(p13;?)$ and $t(1;19)(q23;p12)$; tumor 5, $t(4;5)(p16;q33)$ and $del(9)(q22)$; tumor 8a, $t(3;12)(q21;q24)$. Arrowheads, breakpoints.

single transformed cell (15, 16). Most data favoring a unicellular origin of tumors have been obtained from investigations of hematological or mesenchymal neoplasms. On the other hand, most contradictory results stem from studies of carcinomas. On the basis of clinical and histopathological observations it has been argued that multiple primary cancers are common in several organ systems, including skin, urinary bladder, and upper aerodigestive tract (17–22). The constant exposure to carcinogens at these locations probably induces preneoplastic alterations in an area of epithelium, so-called field cancerization (18), thus markedly increasing the likelihood that several independent neoplastic foci may synchronically or metachronically emerge.

The inferences about the origin of tumors that may be drawn from cytogenetic analyses are likely to be complicated by the fact that most human tumors persist for several years before

they are diagnosed. Kerbel *et al.* (23) have studied the dynamics of clonal evolution during tumor progression by integrating different genetic markers into experimental neoplasms in syngeneic mice. The rate of clonal selection was found to be remarkably high, rapidly creating a homogeneous tumor cell population and a false impression of monocellular origin. Heim *et al.* (24) have used the term cytogenetic convergence to describe this phenomenon, recently exemplified in an *in situ* squamous cell carcinoma of the skin. In short-term cultures from this tumor, 8 unrelated clones were present, but during serial *in vitro* passage the initial heterogeneity was reduced until only 1 of the clones remained (25). The finding of both cytogenetic mono- and polyclonality in BCCs could then, if interpreted within the framework of field cancerization and the competing convergence-divergence tendencies of evolutionary clonal dynamics, be regarded as evidence of a multicellular origin followed by different degrees of clonal selection.

Multiple, unrelated, chromosomally abnormal clones seem to be a feature of other epidermal skin tumors also. A total of 10 such neoplasms have been successfully analyzed cytogenetically (26–32). All had different clonal marker chromosomes, 5 were cytogenetically polyclonal, and most displayed many non-clonal structural chromosome rearrangements. A high percentage of the few squamous cell carcinomas from the larynx, oral cavity, and upper aerodigestive tract that have been cytogenetically investigated have also been polyclonal (6, 33, 34), lending further credence to the notion that at least some carcinomas are of multicellular origin. A note of caution is necessary in this interpretation, however. Although unlikely, one cannot totally disregard the possibility that seemingly different clones in BCCs, and for that matter in the other tumor types, are descendants of a single cell and share a common rearrangement that is detectable only at the molecular level. The chromosome rearrangements must then have occurred during tumor progression, dividing the tumor into a number of subpopulations with different cytogenetic characteristics (35).

It has been hypothesized that tumor progression is speeded up by increased genetic instability (35). This could perhaps be the mechanism behind the high level of nonclonal structural rearrangements that was observed in the majority of the tumors, although the lack of other markers of chromosome fragility (gaps and breaks) makes this interpretation unlikely. Likewise, the assumption that the cells with nonclonal or clonal aberrations are ordinary stroma cells that, through lifelong exposure to different mutagenic agents, *e.g.*, UV light, have accumulated chromosome rearrangements, suffers from several shortcomings. First, stable rearrangements are extremely scarce in dermal fibroblasts (36). Second, using the same short-term culture methods as in BCCs, we have studied both normal skin tissue, admittedly so far only from parts of the body that are not heavily exposed to sunlight, and nonepidermal skin neoplasms without finding nonclonal structural changes.⁴ Third, the non-clonal changes were detected in BCCs from all locations in spite of the differences in sun exposure between, for example, the head and the back. Another possibility would be that in the increased number of chromosomal abnormalities we see the cytogenetic manifestations of field cancerization, *i.e.*, a high frequency of rearrangements in an area of epithelial cells that are not yet fully neoplastic but that also are no longer completely normal.

⁴ F. Mertens, S. Heim, N. Mandahl, B. Johansson, O. Mertens, B. Persson, L. Salemark, J. Wennerberg, N. Jonsson, and F. Mitelman, unpublished data.

ADDENDUM

Since the submission of this manuscript, 2 articles describing cytogenetic findings in basal cell carcinomas have been published. Aledo *et al.* (Int. J. Cancer, 44: 79–83, 1989) found clonal anomalies in 2 of 5 tumors, and in addition nonclonal structural changes were abundant. Scappaticci *et al.* (Cancer Genet. Cytogenet., 42: 309–311, 1989) detected multiple, unrelated clones and many nonclonal rearrangements in a superficial basal cell carcinoma. Thus, although the breakpoints involved in the clonal aberrations differed, the cytogenetic pattern is very similar to our findings.

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