Trisomy 6 in Basal Cell Carcinomas Correlates with Metastatic Potential

A Dual Color Fluorescence In Situ Hybridization Study on Paraffin Sections

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BACKGROUND. Most basal cell carcinomas (BCCs) are indolent lesions; a few become locally aggressive or even metastatic. Little is known about the molecular and genetic alterations in this malignant transformation. Conventional karyotyping in BCC has revealed a high frequency of nonclonal, structural rearrangements, with few cases that show multiple, unrelated, small clones suggestive of a multicellular origin. Trisomy 6 was described recently in a few BCCs, but the biologic significance of the appearance of trisomy 6 in BBCs was not clear.

METHODS. Thirty cases including 4 metastatic, 4 locally aggressive, and 22 conventional nonaggressive BCCs were studied. Fluorescence in situ hybridization (FISH) was performed on 4 μm tissue sections, using α-centromeric enumeration probes for chromosome 6 (SpectrumGreen, Vysis Inc., Downers Grove, IL) and chromosome 4 (SpectrumOrange, Vysis Inc., Downers Grove, IL, used as disomic cell control). Trisomy 6 was semiquantitated within tumor cells and nonneoplastic cells in each case.

RESULTS. Trisomy 6 was identified in all 4 metastatic BCCs within tumor cells and in corresponding BCCs at the primary cutaneous site in 2 of these 4 cases. Two locally aggressive BCCs, 1 of which had preceding radiation exposure, also showed trisomy 6. All nonaggressive BCCs and nonneoplastic cells were disomic for chromosome 6.

CONCLUSIONS. Trisomy 6 has been identified as a cytogenetic aberration representative of tumor cells in aggressive and metastatic BCC. None of the nonaggressive BCCs in this study demonstrated trisomy 6. Acquisition of trisomy 6 by tumor cells in BCC may lead to the emergence of metastatic potential. Additional studies to define the underlying mechanisms may be valuable in preventing aggressive behavior in BCC. ***Cancer* 2001;91:1927–32.** *© 2001 American Cancer Society.*

KEYWORDS: trisomy 6, fluorescence in situ hybridization (FISH), basal cell carcinoma (BCC), metastatic BCC.

Basal cell carcinoma (BCC) in its sporadic form is one of the most common neoplasms occurring in the white population. Most BCCs are slow-growing, indolent lesions cured by complete excision. However, a few BCCs (4%) recur regardless of the mode of therapy.[1] BCC can metastasize, like any other carcinoma, although in less than 0.1% of cases[2] BCCs at risk for metastasis are typically large, locally aggressive, recalcitrant lesions on the head and neck, with regional lymph nodes as the most common metastatic site.[3]

Histologically, the tumor is comprised of islands of basaloid cells, often with a palisaded edge, surrounded by a characteristic stroma. Little is known, however, about genetic or molecular alterations in BCC, particularly those underlying the evolution of a truly malignant

TABLE 1
Clinicopathologic Features of 22 Nonaggressive Basal Cell Carcinomas

No.	Age/Gender	Site	Size[a]	Invasion	Histology	FISH
1	75 M	Midchest	0.4	SD	Superficial	No +6
2	89 F	Lower back	0.4	SD	Superficial	No +6
3	89 M	Forehead	0.5	SD	Superficial	No +6
4	55 F	Ear	1.9	DD	Nodular	No +6
5	69 M	Cheek	0.4	SD	Adenoid	No +6
6	70 M	Upper chest	0.9	SD	Superficial	No +6
7	65 F	Upper lip	0.2	SD	Solid	No +6
8	72 M	Preauricular	0.4	DD	Solid	No +6
9	76 M	Upper arm	1.0	DD	Adenoid	No +6
10	68 M	Shoulder	0.4	SD	Superficial	No +6
11	87 F	Neck	0.3	DD	Nodular	No +6
12	86 M	Upper lip	0.3	SD	Nodular	No +6
13	34 F	Upper back	1.0	DD, SC	Nodular	No +6
14	62 M	Forehead	0.5	SD	Nodular	No +6
15	79 M	Chin	0.5	SD	Nodular	No +6
16	75 M	Nasal ala	0.4	SD	Nodular	No +6
17	95 M	Midback	0.9	DD, SC	Nodular	No +6
18[b]	80 M	Forehead	0.5	DD	Infiltrating	No +6
19	74 F	Forearm	1.1	DD	Infiltrating	No +6
20[b]	88 F	Not known	0.6	DD	Morphea	No +6
21	89 F	Lower lip	0.9	DD	Nodular	No +6
22	90 M	Neck	0.4	DD	Nodular	No +6

FISH: fluorescence in situ hybridization; SD: superficial dermis; DD: deep dermis, SC: subcutis, +6: trisomy 6.

[a] Size in cm.

[b] No hybridization.

behavior in BCC. Point mutations in the p53 gene have been observed both in BCC and in nonneoplastic sun -exposed skin[4] Loss of heterozygosity for loci on chromosome 9q, where the gene for hereditary BCC is located, has been observed in sporadic BCC.[5] At the present time, however, there is no definite evidence of consistent cytogenetic or molecular alteration specific to sporadic BCCs. The problems associated with solid tumor cytogenetics (insufficient mitoses, fibroblast overgrowth, poor chromosome banding) are even more pertinent in this slow-growing tumor with a prominent stromal component.[6] Studies of short-term BCC cultures[6–12] (harvested at 1–2 weeks) repeatedly have revealed a high frequency of nonclonal structural rearrangements with multiple, small, unrelated, pseudodiploid clones within a single tumor population. In addition, conventional karyotypic analyses of nonneoplastic sun-exposed skin, both in the vicinity of and away from the tumor, have revealed similar cytogenetic changes, albeit in a lesser proportion of cases compared with BCC.[13,14]

In a cytogenetic study of 22 BCCs, Jin et al.[15] reported t(9;16)(q22;p13) as one of multiple, small clones in 3 of 22 BCCs examined by conventional methods following short-term culture. It is well recognized that the results of conventional karyotyping in solid tumors should be interpreted with caution,[16,17] because it is difficult to be certain of the nature of the cell that is demonstrating an aberration. A number of questions still remain: 1) Are the observed cytogenetic aberrations in BCC truly representative of the tumor cell population and, if so, are they of pathogenetic significance? 2) Do these aberrations have any correlation with histologic type? 3) Is there a cytogenetic or molecular event that underlies the evolution of metastatic potential in BCC?

To avoid overgrowth by fibroblasts, efforts to analyze direct preparations of BCCs (harvested at 24–48 hrs) have met with little success.[18] Casalone et al[19] found trisomy 6 in 5 of 25 direct preparations of BCC; however, the significance of trisomy 6 in their BCC cases was not clear.

The fluorescence in situ hybridization (FISH) technique has the advantage of examining nondividing cells and, when performed on paraffin sections, permits correlation of morphology with cytogenetic findings. In the current study, trisomy 6 was demonstrated as a cytogenetic aberration truly representative of the tumor cells in BCC, and its presence was correlated with aggressive biologic behavior in BCC.

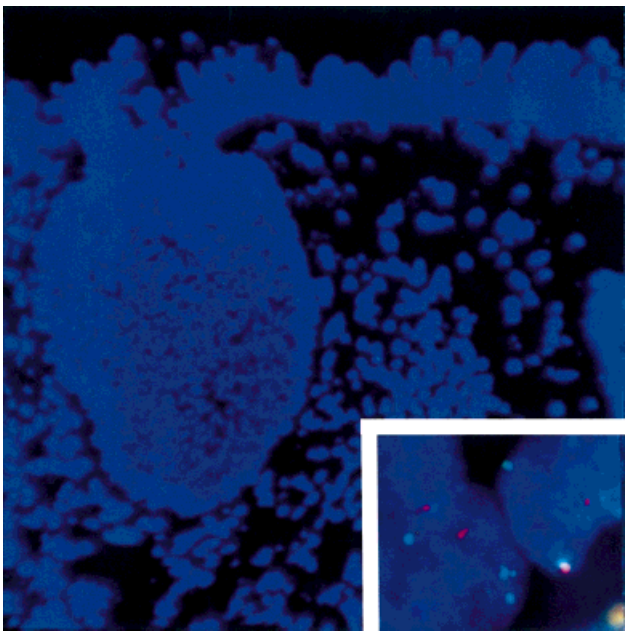

FIGURE 1. Nonaggressive superficial BCC (Table 1, Case 1) showing architectural morphology by nuclear counterstain (DAPI, original magnification, ×150). *Inset:* Cells with normal diploid signals for centromere 4 and 6 (CEP 4 SpectrumOrange/CEP 6 SpectrumGreen/DAPI, original magnification, ×1500).

MATERIALS AND METHODS

Patient Population

The surgical pathology files of Roswell Park Cancer Institute were searched from 1994–7 for metastatic and locally aggressive BCCs. Four cases in each category were retrieved and a review of the medical records was performed. Cases included as nonaggressive BCC ($n = 22$) were sporadic, selected at random, and included various histologic subtypes (Table 1). Locally aggressive BCC ($n = 4$, 2 of which were synchronous lesions in 1 patient; Table 2) were defined clinically based on size, depth of invasion, and difficulty in management. In each case, a representative formalin-fixed, paraffin-embedded, primary or metastatic tumor block was selected by examination of the original hematoxylin and eosin (H&E) sections while confirming of the pathologic diagnoses. Paraffin blocks from the corresponding BCC at the primary cutaneous site were available in 2 of 4 metastatic cases (Table 3, Cases 1 and 4). Two additional blocks were retrieved from one metastatic BCC (Table 3, Case 3), corresponding to nonneoplastic skin and benign lymph nodes from the same surgical resection as the metastatic lymph node.

Fluorescence In Situ Hybridization

Four μm sections were prepared for FISH by using a paraffin pretreatment reagent kit (Vysis Inc., Downers Grove, IL), according to the manufacturer's instructions. FISH was performed using α-centromeric enumeration probes for chromosome 4 (D4Z1, CEP 4 SpectrumOrange, Vysis Inc.) and chromosome 6 (D6Z1, CEP 6 SpectrumGreen, Vysis Inc. simultaneously. Chromosome 4 was selected as an internal control for disomic cells based upon the absence of numeric aberrations of chromosome 4 in reviewing previous cytogenetic studies of BCC. Codenaturation of probe and target DNA was accomplished at 85 °C for 1 minute, followed by overnight hybridization at 42 °C, by using the HYBrite system (Vysis Inc). Following posthybridization washes 0.4× in saline-sodium citrate buffer (SSC)/SSC/0.1% NP-40 at 75 ± 1 °C for 2 min, followed by 2× in SSC/0.1% NP-40 at room temperature for 1 min), a nuclear counterstain (DAPI II, Vysis Inc.) was applied, and sections were examined on a Nikon Optiphot-2 (Nikon USA) epifluorescence microscope. Each section was scanned (×150, DAPI filter) for architectural morphology (Fig. 1) with visual quantitation of the probe signals at higher magnification

TABLE 2
Clinicopathologic Features of 4 Aggressive Nonmetastatic Basal Cell Carcinomas

No.	Age/Gender	Site	Size[a]	Invasion	Histology	FISH	Treatment	Follow-up
1[b]	51 M	Chin, neck, and upper chest	19.0	Skeletal muscle	Nodular	Rare tumor cells +6	Surgery + RT	AWNED at 8 mos
2[b]	52 M	Forehead	1.0	Skeletal muscle	Nodular	10% tumor cells +6	Surgery	AWNED at 5 mos
3	54 M	Ear[c]	1.5	Cartilage	Infiltrating	No +6	Surgery	AWNED at 18 mos
4	65 M	Behind ear[c]	2.0	Bone	Infiltrating	No +6	Surgery	AWD at 4 mos

FISH: fluorescence in situ hybridization; +6: trisomy 6; RT: radiotherapy; AWD: alive with disease; AWNED: alive with no evidence of disease.

[a] Size in cm.

[b] Same patient.

[c] Recurrence.

TABLE 3
Clinicopathologic Features of 4 Metastatic Basal Cell Carcinomas

No.	Age/ Gender	Primary basal cell carcinoma				Metastases at time of study		Follow-up
		Site	Size[a]	Histology	FISH	Site	FISH	
1	64 M	Neck[b]	5.0	Infiltrating	Rare tumor cells +6	1 of 2 regional LN at 2 yrs	75% tumor cells +6	T4 metastases at 1 yr, DOD at 2 yrs
2	69 M	Ear helix and face	NA	NA	NA	Supraorbital (intradermal)	20% tumor cells +6	Metastases of lung at 2 mos, hospice at 6 mos
3	74 M	Shoulder	14.5	Adenoid	NA	2 of 28 axillary LN at 2 yrs	75% tumor cells +6	AWNED at 4 mos
4	72 M	Ear[b]	10.0	Infiltrating and nodular	50% tumor cells +6	1 of 2 regional LN at 5 yrs	60% tumor cells +6[c]	AWD at 1 yr

FISH: fluorescence in situ hybridization; NA: not available for study; +6 = trisomy 6; LN = lymph node; T4: thoracic 4 vertebra; DOD: dead of disease; AWNED: alive with no evidence of disease; AWD: alive with disease.

[a] Size in cm.

[b] Recurrence.

[c] 5% cells with tetrasomy 6.

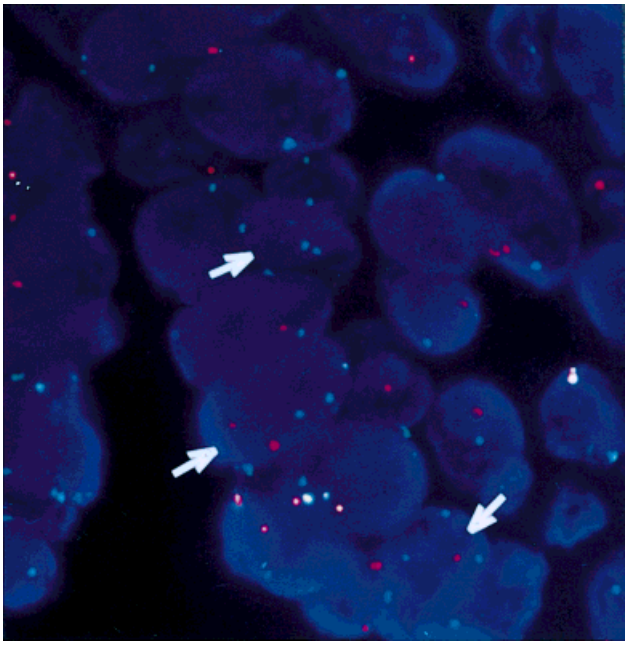

FIGURE 2. Locally aggressive BCC, nodular type (Table 2, Case 2) showing trisomy 6 (arrows) in palisaded edge of tumor nodule (CEP 4 SpectrumOrange/ CEP 6 SpectrumGreen/DAPI, original magnification ×1500).

(×1500 oil, single, dual, and triple bandpass filters). In situ hybridization was considered successful if easily visible probe signals were present in most or all nuclei. At least 20 high-power fields (×1500) or the entire section, if small, were studied in areas with minimal nuclear overlap and clearly defined nuclear outlines. When present, the percentage of tumor cells showing trisomy 6 was estimated. Non-neoplastic cells, including surrounding stromal cells, benign epithelium, skin appendages, and lymphocytes were studied in each case. The images were captured using a monochrome CCD camera (Cohu, Inc., San Diego, CA) and image analysis software (MacProbe, Perceptive Scientific Instruments Inc., League City, TX).

RESULTS

The clinicopathologic features of 22 conventional nonaggressive, 4 aggressive, and 4 metastatic BCCs are presented in Tables 1, 2, and 3 respectively. In situ hybridization was accomplished in all cases except 2 nonaggressive BCCs. Numeric abnormalities of chromosome 4 were not observed in any case. Trisomy 6 was not identified in any of the 20 nonaggressive BCCs (example in Fig. 1) in this study. Trisomy 6 was present in 2 aggressive BCCs in one patient (Table 2, Cases 1 and 2); in rare tumor cells in the initial BCC (Table 2, Case 1), and in a single nodular focus within the synchronous tumor, overall constituting approximately 10% of tumor cells (Table 2, Case 2, Fig. 2).

Trisomy 6 was identified in tumor cells within metastatic sites from all 4 patients with metastatic BCC (Table 3). The 3 lymph node metastatic foci demonstrated trisomy 6 in a high proportion (50–80%) of

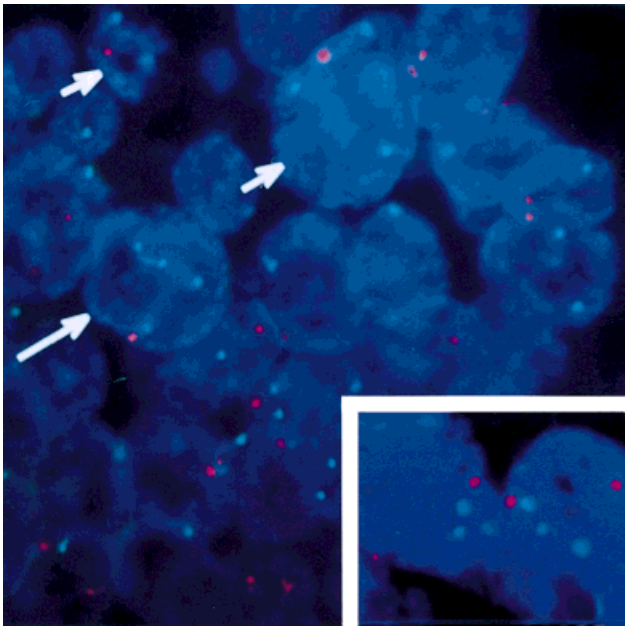

FIGURE 3. Metastatic BCC in regional lymph node (Table 3, Case 4) showing tumor cells with trisomy 6 (small arrows) and tetrasomy 6 (large arrow). *Inset:* Trisomy 6 in corresponding BCC at primary cutaneous site (CEP 4 SpectrumOrange/CEP 6 SpectrumGreen/DAPI, original magnification, ×1500).

tumor cells, whereas the intradermal metastatic lesion had trisomy 6 in approximately 20% of tumor cells. In 1 lymph node with metastatic carcinoma (Table 3, Case 4), about 5% of randomly distributed tumor cells also demonstrated tetrasomy 6 (Fig. 3). In the 2 corresponding BCCs at the primary cutaneous site (Table 3, Cases 1 and 4), trisomy 6 was observed in rare and about 50% of tumor cells, thus suggesting a direct derivation of the metastatic tumor from the primary site. In all cases, trisomy 6 was absent in stromal cells adjacent to tumor and in cells of benign cutaneous epithelium and appendages. Nonneoplastic cells in additional sections of skin and benign lymph nodes in 1 metastatic BCC (Table 3, Case 3) were negative for trisomy 6.

DISCUSSION

Most data in favor of a unicellular origin for neoplasms is based upon investigation of hematologic and mesenchymal neoplasms[20] In comparison, little is known about karyotypic abnormalities in tumors of epithelial origin[21,22] The karyotypic pattern in most epithelial neoplasms is characterized by multiple and nonspecific aberrations, in contrast to hematologic malignancies, which are characterized by simple and disease-specific abnormalities.[23] The cytogenetic and molecular aberrations reported in BCC, and their similarity with those observed in nonneoplastic skin indicate an initial polyclonal process, perhaps a reflection of cumulative DNA damage from exposure to sunlight. In light of the frequent overgrowth of fibroblasts in BCC cell cultures, these observed changes may not even be representative of the tumor cell population in BCC[11]

In this study, trisomy 6 was identified in tumor cells from metastatic foci in all 4 patients studied and in recurrences of BCC at the primary cutaneous site in 2 of these patients, thus suggesting a direct derivation of tumor at the metastatic site from the primary site. None of the 20 nonaggressive BCCs in this study showed polysomy of chromosome 6. Evidence of trisomy 6 localization in the stromal cells or other nonneoplastic cells adjacent to the epithelial component of BCC was not identified in any of these cases. Of the locally aggressive BCCs studied, trisomy 6 was observed in 2 synchronous BCCs in the same patient. The initial tumor was a long standing, large, 19 cm BCC on the patient's neck and upper chest, which was treated by radiation therapy. While on treatment, a second, small BCC developed on the patient's forehead. This subsequent BCC demonstrated trisomy 6 in 1 nodular focus, predominantly at the palisaded edge of the tumor (Fig. 2), whereas the nonneoplastic, cutaneous epithelium was disomic for chromosome 6.

In the abstract by Casalone et al.,[19] there was no discussion of the significance of trisomy 6, which was detected in 5 of 25 BCC cases by the direct method. In this study, the association of trisomy 6 with aggressive biologic behavior in BCC was demonstrated. A review of numeric abnormalities of chromosome 6 in neoplastic diseases indicates that trisomy 6 is associated with increased aggressiveness in a few neoplasms. It has been correlated with a higher grade of non-Hodgkin lymphoma,[24] and isochromosome 6p (functional trisomy 6p) has been linked to tumor progression in retinoblastoma.[25] In basal cell carcinomas, trisomy 6 may be an initial or primary event occurring in a few cases rendering the tumor cell more aggressive, with acquisition of metastatic potential by a hematogenous or a lymphatic route. The etiologic agents that trigger this genetic change are not yet well defined. One predisposing factor may be radiation exposure, which was identified in 1 patient with a trisomy 6 positive locally aggressive BCC in this study.

In conclusion, trisomy 6 is confirmed as a nonrandom aberration occurring only within tumor cells, and not stromal cells, in BCC. In this study, trisomy 6 is present only in BCCs that display aggressive biologic behavior. Acquisition of trisomy 6 by tumor cells in BCC may play a significant role in the emergence of

metastatic potential. Additional studies to determine the mechanism underlying this genetic event may provide valuable practical insight in preventing the emergence of aggressive behavior in this conventionally indolent neoplastic process.

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