Tumorigenesis and Neoplastic Progression

USP6 and *CDH11* Oncogenes Identify the Neoplastic Cell in Primary Aneurysmal Bone Cysts and Are Absent in So-Called Secondary Aneurysmal Bone Cysts

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Aneurysmal bone cyst (ABC) is a locally recurrent bone lesion that has been regarded as a reactive process. Recently, a neoplastic basis in primary ABC was evidenced by demonstration of clonal chromosome band 17p13 translocations that place the *USP6* (*TRE2* or *TRE17*) oncogene under the regulatory influence of the highly active *CDH11* promoter. Herein, we report *CDH11* and/or *USP6* rearrangements in 36 of 52 primary ABCs (69%), of which 10 had *CDH11-USP6* fusion, 23 had variant *USP6* rearrangements without *CDH11* rearrangement, and three had variant *CDH11* rearrangements without *USP6* rearrangement. *USP6* and *CDH11* rearrangements were restricted to spindle cells in the ABC and were not found in multinucleated giant cells, inflammatory cells, endothelial cells, or osteoblasts. *CDH11* and *USP6* rearrangements did not correlate with recurrence-free survival, or with other clinicopathological features. *CDH11* and *USP6* rearrangements were not found in any of 17 secondary ABC associated with giant cell tumor, chondroblastoma, osteoblastoma, and fibrous dysplasia. These findings demonstrate that primary ABC are mesenchymal neoplasms exhibiting *USP6* and/or *CDH11* oncogenic rearrangements. By contrast, secondary ABC lack *CDH11* and *USP6* rearrangements, and although morphological mimics of primary ABC, appear to represent a non-specific morphological pattern of a diverse group of non-ABC neoplasms. (*Am J Pathol 2004, 165:1773–1780*)

Aneurysmal bone cyst (ABC) is an intriguing bone lesion with the potential for local recurrence that has been regarded as a reactive process since its initial description in 1942 by Jaffe and Lichtenstein.[1] Different theories have been proposed for the pathogenesis of ABC, and among the most widely accepted has been that a local circulatory abnormality leads to an increased venous pressure and resultant dilation of the vascular network.[2–5] The reactive nature of ABC has also been suggested by the fact that a variety of benign and malignant bone neoplasms,[5–8] including giant cell tumor of bone, chondroblastoma, osteoblastoma, fibrous dysplasia, and osteosarcoma may contain areas within the lesion that closely mimic ABC histologically. For these cases, the term "secondary ABC" has been coined.[3,7] However, it is unclear whether primary and secondary ABC have a similar pathogenesis, or whether secondary ABC might rather be a common morphological pattern of growth that occurs as a non-specific phenomenon in a variety of primary bone tumors.

The purported reactive nature of ABC was refuted by the work of Panoutsakopoulos et al,[9] which demonstrated chromosomal translocation t(16;17)(q22;p13) as a recurrent cytogenetic abnormality in primary ABC. Subsequently, other groups confirmed 17p13 rearrangement as a frequent cytogenetic aberration in primary ABC.[10–15] Recently, we extended these studies by showing that the

t(16;17)(q22;p13) fuses the promoter region of the osteo-blast cadherin 11 gene (*CDH11*) on chromosome 16q22 to the entire coding sequence of the ubiquitin protease *USP6* gene (also known as *TRE2* or *TRE17*) on chromosome 17p13, suggesting that the pathogenesis of many primary ABC involves up-regulation of *USP6* transcription.[16]

In this study we determined the frequency and the clinicopathologic consequences of *CDH11* and *USP6* genetic abnormalities in 52 primary ABC, and we also used these molecular markers to evaluate the pathogenetic relationship or lack thereof between primary and secondary ABC. Further, we identify the neoplastic cell in primary ABC by determining which cell components contained the genetic rearrangements.

Materials and Methods

Tumor Samples

Fifty-two primary ABC and 17 secondary ABC were histologically characterized according to established criteria.[8] Metaphase preparations were available from eight of these cases, including two for which the cytogenetic findings have been reported previously (cases 19 and 29).[16] Frozen tissue was available from 16 cases.

Fluorescence in Situ Hybridization (FISH)

BAC clones were obtained from Children's Hospital Oakland Research Institute (Oakland, CA) and Research Genetics (Huntsville, AL). BAC minicontigs were assembled based on genomic mapping and sequence data from the Human Genome Working Draft. Minicontigs telomeric and centromeric to the *USP6* locus were USP6. T (BACs RP11–124C16, RP11–111I16, and RP11–177H5) and USP6. C (CTD-2367F23 and RP11–457I18), respectively. Those telomeric and centromeric to *CDH11* were CDH11. T (RP11–137A18, RP11–631H23, and RP11–351A20) and CDH11. C (RP11–615M9, RP11–730A21, and RP11–76J1), respectively. BAC DNA isolations and labeling were performed as described previously.[16]

FISH was performed using 4-μm paraffin-embedded tissue sections which were deparaffinized in xylene (3 × 10 minutes), dehydrated twice in 100% ethanol for 2 minutes, and treated with 100 mmol/L Tris and 50 mmol/L EDTA (pH 7.0) for 15 minutes at 93°C. Tissue sections were then rinsed once in 1X PBS and protein digested with Digest All-3 (Zymed, San Francisco, CA). After briefly washing in 1X PBS, the slides were sequentially dehydrated in alcohol (70%, 85%, 95%, and 100%), and air-dried for 1 hour at room temperature. Tissue sections were denatured at 75°C for 2 minutes and BAC probe hybridization was carried out overnight in a humidified chamber at 37°C. Tissue sections were then washed in 0.5X SSC for 5 minutes at 73°C and treated with CAS block (Zymed) for 10 minutes. Probe detection was performed using FITC-anti-digoxigenin (1:500) and Alexa Fluor 594-streptavidin (1:500) (Molecular Probe, Eugene, OR)[17] for 30 minutes. Slides were then mounted in VECTASHIELD mounting medium with 1.5 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI). ABC were scored as positive for gene rearrangement if more than 5% of cells showed splitting apart of the flanking FISH probes. If fewer than 20% of cells showed rearrangement, the findings were corroborated by repeating the FISH assay in another section from the same paraffin block.

RNA Isolation and RT-PCR

RNA was isolated from frozen tumors after mechanical homogenization and overnight incubation in Trizol (Invitrogen, Carlsbad, CA) at 4°C. RNA reverse transcription into cDNA was performed using the GeneAmp RNA PCR Kit (Applied Biosystems, Foster City, CA) for 2 hours at 42°C using random hexamers. RNA isolation from paraffin sections were performed according to a previously described protocol.[18]

PCR reactions were performed using the Takara Ex *Taq* kit with the following parameters for 35 cycles: denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 1 minute. PCR primers for RT-PCR evaluation of the *CDH11-USP6* fusion oncogene were *CDH11*+71F (5′-CGCCGCTGACTTGT-GAAT-3′) and *USP6*+1781R (5′-CTCGGTGTCCCTTGT-CATACTT-3′). Evaluation of low-abundance fusion transcripts in tumors where the first round PCR was negative was performed by nested PCR using primers *CDH11*+83F (5′-GTGAATGGGACCGGGACT-3′) and *USP6*+1736R (5′-CAGGAGCGGAAGGACATACTTA-3′) at the same cycling parameters as above for 25 cycles.

Statistical Analysis

Statistical analyses were performed with the S-PLUS 6.0 software package (Insightful Corp., 2001). The Fisher exact test was used to evaluate associations among categorical variables. The Mann-Whitney *U*-test was used to evaluate associations between categorical and continuous variables. Recurrence-free survival was calculated using the Kaplan-Meier product-limit method, and univariate survival analyses were calculated using the log-rank test. Multivariate survival analyses were performed using the Cox multivariate regression model. Proportionality assumption was evaluated using Kaplan-Meier and log-minus-log survival curves. Reported *P* values were two-sided and statistical significance was set at $P \leq 0.05$.

Results

Overall Clinical and Morphological Features of Primary ABC

Clinical features are summarized in Table 1. Median patient age at diagnosis was 14 years (range, 2 to 42 years), and the genders were equally represented (27 females and 25 males). The most frequent primary sites were tibia ($n = 11$), femur ($n = 8$), fibula ($n = 7$), vertebra ($n = 5$), and humerus ($n = 4$). Thirty-nine tumors arose in peripheral locations, whereas 13 were central. Median tumor

Table 1. Clinical Features of Primary ABC

Case	Age (years)	Sex	Location*	Size (cm)	Treatment	Follow-up (mo)	Recurrence	Time for recurrence (mo)
1	2	M	Femur	4	Curettage	73	Y	30
2	2	F	Femur	N/A	Curettage	4	N	
3	5	M	Pubis	5	Curettage	9	N	
4	7	M	Femur	4	Curettage	9	Y	5
5	7	F	Tibia	5	Curettage	1	N	
6	8	F	Tibia	5	Curettage	54	N	
7	8	F	Fibula	2	Curettage	N/A	N/A	
8	10	M	Calcaneous	4	Curettage	25	N	
9	10	M	Tibia	N/A	Curettage	108	N	
10	10	M	Femur	N/A	N/A	N/A	N/A	
11	11	F	Mandible	1	Excision	N/A	N/A	
12	11	M	Vertebra	5	Curettage	18	Y	2
13	11	M	Phalanx	1	Curettage	9	Y	6
14	11	M	Mandible	3	Curettage	21	Y	11
15	11	F	Humerus	3	Curettage	94	N	
16	11	M	Femur	6	Curettage	25	Y	6
17	12	M	Tibia	4	Curettage	30	N	
18	12	M	Fibula	4	Curettage	N/A	N/A	
19[†]	13	F	Tibia	4	Curettage	2	N	
20	13	M	Femur	4	Curettage	18	N	
21	13	M	Femur	7	Curettage	70	N	
22	13	F	Fibula	3	Curettage	18	N	
23	13	F	Phalanx	1	Curettage	30	N	
24	14	M	Tibia	N/A	N/A	N/A	N/A	
25	14	F	Clavicle	2	Curettage	21	Y	21
26	14	M	Fibula	4	Curettage	8	N	
27	14	F	Tibia	2	Curettage	51	Y	12
28	14	F	Radius	1	Curettage	24	Y	7
29[†]	15	F	Pubis	4	Curettage	28	Y	6
30	15	F	Fibula	5	Curettage	49	Y	6
31	15	F	Vertebra	4	Excision	60	N	
32	15	F	Tibia	N/A	N/A	N/A	N/A	
33	16	F	Vertebra	6	Excision	12	N	
34	16	F	Tibia	2	Curettage	6	N	
35	17	M	Vertebra	5	Excision	12	Y	10
36	17	F	Mandible	4	Curettage	22	N	
37	17	F	Tibia	5	Curettage	46	Y	5
38	20	F	Tibia	4	Curettage	7	N	
39	26	F	Phalanx	4	Excision	33	N	
40	27	M	Humerus	3	Curettage	5	N	
41	27	F	Clavicle	2	Curettage	6	Y	6
42	29	F	Calcaneous	N/A	Curettage	44	Y	35
43	32	M	Talus	3	Curettage	12	N	
44	42	M	Femur	3	Curettage	12	N	
45	6	M	Humerus[s]	5	Curettage	31	N	
46	7	F	Ulna[s]	2	Curettage	N/A	N/A	
47	8	M	Clavicle[s]	2	Curettage	78	Y	5
48	9	F	Vertebra[s]	3	Excision	18	N	
49	9	M	Calcaneous[s]	4	Curettage	18	Y	6
50	15	F	Fibula[s]	4	Curettage	4	N	
51	18	M	Fibula[s]	3	Curettage	1	N	
52	8	M	Shoulder[st]	8	Excision	N/A	N/A	

*, s, denotes solid variant; st, denotes soft tissue tumor; N/A, not available.
[†], previously reported cases.[16]

size was 4 cm (range, 1 to 8 cm). Treatment information was available for 49 patients, of whom 42 (86%) were treated by curettage and seven by local excision. No patient received radiotherapy. Clinical follow-up was available for 44 patients, of whom 17 (39%) had a local recurrence after a median interval of 35 months (range, 2 to 35 months). Two patients had more than one recurrence.

All ABC were reviewed histologically and were classified according to contemporary criteria.[8] Forty-five cases exhibited classic histology, featuring cavernous or slit-like hemorrhagic spaces surrounded by fibrous septa containing spindle cells and occasional osteoclast-like multinucleated giant cells. Osteoid formation with osteoblastic rimming was observed in all cases, and matrix calcification was observed in 17 cases (33%). Seven cases were solid variants of ABC, featuring a prominent solid growth and minimal or no cystic formation but otherwise histologically indistinguishable from the classic ABC.

Table 2. Molecular Genetic Features of Primary ABC

Case*	FISH rearrangement			RT-PCR
	USP6	CDH11	%	CDH11-USP6
1	−	−	0	−
2	+	−	18	
3	+	+	58	+
4	+	−	32	−
5	+	−	40	−
6	−	−	0	
7	+	+	60	+
8	−	−	0	−
9	+	−	52	
10	+	+	10	+
11	+	+	44	+
12	+	−	28	
13	+	−	82	−
14	−	−	0	
15	−	−	0	
16	+	−	60	−
17	+	−	52	
18	−	−	0	
19†	+	+	36	+
20	+	+	27	+
21	−	−	1	−
22	−	−	0	
23	+	−	20	
24	+	−	33	−
25	+	−	35	−
26	+	−	80	−
27	−	+	30	−
28	−	−	0	
29†	+	+	56	+
30	+	−	81	−
31	+	+	68	+
32	−	−	0	
33	+	+	32	+
34	+	−	60	
35	+	−	34	−
36	+	−	32	
37	−	−	0	−
38	−	−	0	
39	+	−	16	−
40	−	−	0	−
41	−	−	0	
42	−	−	0	
43	+	−	8	
44	−	+	50	
45ˢ	+	−	39	
46ˢ	+	−	35	
47ˢ	+	−	58	
48ˢ	−	+	30	
49ˢ	+	−	54	
50ˢ	−	−	1	−
51ˢ	+	+	7	+
52ˢᵗ	+	−	38	

*, s, denotes solid variant; st, denotes soft tissue tumor.
†, previously reported cases.[16]

Molecular Cytogenetics and Molecular Genetics of Primary and Secondary ABC

FISH analyses in 52 primary ABC showed that 36 (69%) had CDH11 and/or USP6 locus rearrangement (Table 2). Of these, 10 cases (28%) exhibited rearrangement of both loci, and a fusion transcript CDH11-USP6 was confirmed by RT-PCR in each of these cases. Notably, 23 ABC featured USP6 rearrangement without associated CDH11 rearrangement, consistent with variant USP6 activation mechanisms (Table 2). Only three ABC had CDH11 rearrangement without associated USP6 rearrangement (Table 2). CDH11-USP6 RT-PCR validations were performed in a representative group of the ABC with FISH rearrangements of USP6 only, CDH11 only, or neither USP6 or CDH11, and each of these tumors lacked the CDH11-USP6 fusion transcript (Table 2). Generally, the CDH11 and USP6 rearrangements appeared to be genomically balanced, although two ABCs had an unbalanced USP6 rearrangement with loss of chromosomal material telomeric to USP6, as evidenced by deletion of the FISH probe in that region. Another ABC had an unbalanced rearrangement of the CDH11 locus with loss of chromosome material centromeric to CDH11. Among seven solid variants of ABC, six cases had CDH11 or USP6 loci rearrangements (Table 2).

FISH and RT-PCR analysis were also performed in 17 secondary ABC, of which eight were associated with giant cell tumors, five with chondroblastomas, three with osteoblastoma, and one with fibrous dysplasia. In these lesions, both the primary tumor component as well as the secondary ABC lacked CDH11 and USP6 rearrangements by FISH (Figure 1, D and E), and lacked CDH11-USP6 fusion transcripts by RT-PCR.

Identification of Cell Types with USP6 Rearrangement

In primary ABC (Figure 1A) with USP6 or CDH11 rearrangement, the percentage of abnormal cells varied from 7% to 82% (median, 27%). These cells were usually spindled, wavy, or oval (Figure 1B), and were indistinguishable morphologically from surrounding spindle cells that lacked USP6 rearrangement. The cytogenetically abnormal cells were generally scattered diffusely throughout the lesion and were often grouped in small or large clusters adjacent to multinucleated giant cells (Figure 1C). USP6 and CDH11 rearrangement were never seen in the multinucleated giant cells, inflammatory cells, endothelial cells, and in the metaplastic bone-associated osteoblasts, corroborating their non-neoplastic nature. In addition, ABC cyst wall lining cells did not have USP6 or CDH11 locus rearrangement. Ki-67 antigen (MIB-1) median labeling index in ABC was 7% (range, 1 to 30%), and there was no association between the presence of USP6 locus rearrangement and MIB1 labeling index ($P = 0.47$).

Clinicopathologic Correlations of USP6 and CDH11 Rearrangements in ABC

Clinical correlates were compared in primary ABC with molecular aberrations (USP6 or CDH11 rearrangement) versus those lacking demonstrable USP6 or CDH11 alterations. Patients with ABC with and without the molecular aberrations did not differ in age at diagnosis ($P = 0.27$), gender ($P = 0.38$), tumor size ($P = 0.99$), or location (central versus peripheral; $P = 0.30$). In addition,
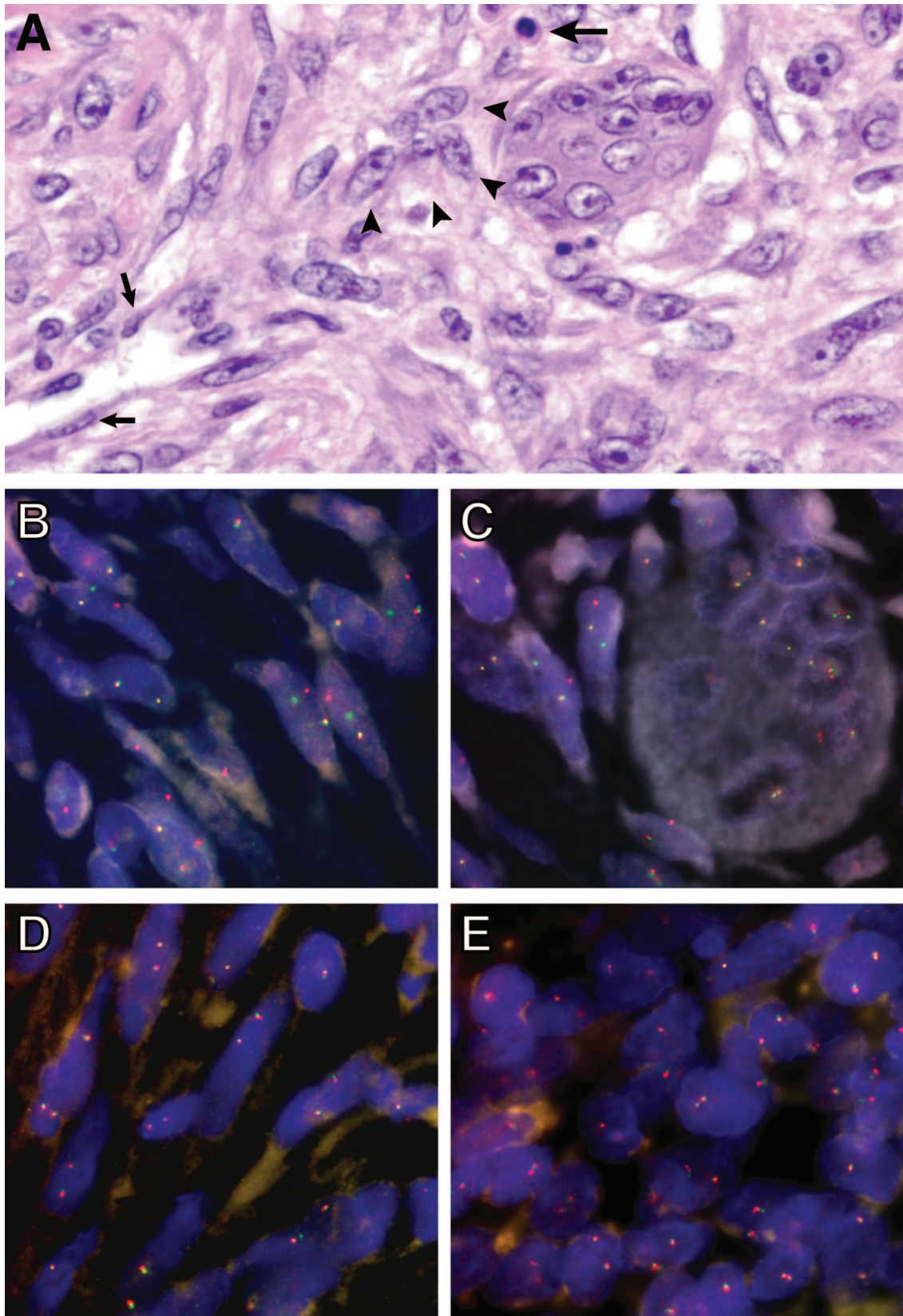
Figure 1. Histological and molecular cytogenetic features in ABC. **A:** Histology (H&E) of primary ABC shows an inflammatory cell (**large arrow**), endothelial cells (**small arrows**), and a cluster of ovoid-to-spindled neoplastic cells (**arrowheads**) adjacent to a multinucleated giant cell. **B–E:** Dual-color "split-apart" *USP6* fluorescence *in situ* hybridization (FISH) was performed in paraffin-embedded tissues using probes on the centromeric (green) and telomeric (red) sides of the *USP6* locus. Splitting apart of a green-red probe signal indicates *USP6* rearrangement. *USP6* rearrangement is seen in spindle-shaped cells in a primary ABC (**B**) and in spindle cells clustered around a multinucleated giant cell (**C**), whereas the multinucleated cell lacks *USP6* rearrangement. A secondary ABC occurring in association with a chondroblastoma lacks *USP6* rearrangement in the ABC (**D**) and chondroblastoma (**E**) components.
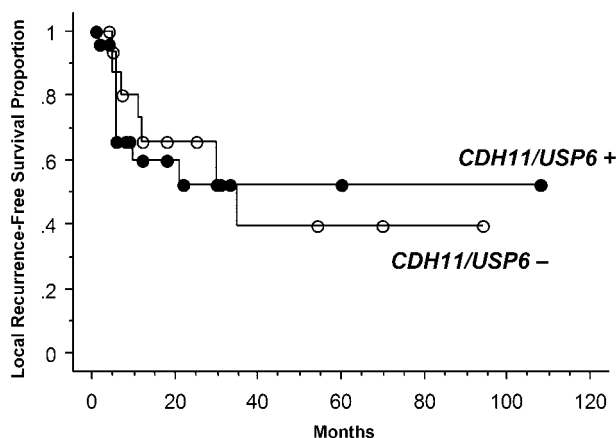
Figure 2. Kaplan-Meier curves for local recurrence-free survival in primary ABC stratified according to the presence or absence of *CDH11* and *USP6* loci rearrangements.

there was no difference in local recurrence-free survival after a median follow-up of 28 months (range, 1 to 108 months) (Figure 2 and Table 3). Cox multivariate regression analysis confirmed these findings and showed that only central tumor location was associated with an increased risk for local recurrence after adjusting for age, gender, tumor size, type of treatment (curettage or complete excision), and molecular aberrations (Table 3).

Discussion

ABC is a locally destructive bone lesion that has been regarded as a reactive process since its initial description more than 60 years ago.[1,2,19] ABC generally occurs during the first two decades of life and females are affected slightly more often than males.[19] The most frequent anatomical locations of ABC are the metaphyses of long bones, especially distal femur, proximal tibia, and posterior vertebral bodies.[3,19,20] Radiologically, ABC presents typically as an eccentric and expansile lytic lesion that is often associated with a sclerotic margin.[19,21] Clinically, ABC is characterized by a propensity to local recurrence,[8,19] especially during the first 2 postoperative years.[19] ABC is most often treated with curettage followed by bone grafting;[3] wide excision is less often used,

Table 3. Clinicopathogic Correlates with Local Recurrence-Free Survival in ABC

Variables	Univariate analysis *p* value	Multivariate analysis *p* value	HR	95% CI
Age >= 14 years	0.62			
Male Sex	0.56			
Central location	0.15	0.03	3.2	1.1–9.0
Tumor size >= 4	0.54			
CDH11 or *USP6* abnormalities	0.89			
Curretage X excision	0.25	0.1	6	0.7–49

Likelihood ratio for the model: p = 0.05.
p to exclude in the multivariate model: >0.10.

and radiation and amputation are reserved only for exceptional cases.

Histologically, ABC is characterized by hemorrhagic cystic and cavernous spaces of varying size surrounded by fibrous septa or more solid areas composed of mitotically active spindle or ovoid cells intermixed with inflammatory cells, scattered osteoclast-like multinucleated giant cells, extravasated red blood cells, and numerous capillaries. The histological growth is reminiscent of early granulation tissue. Trabecular osteoid formation with active osteoblasts and a characteristic matrix calcification with chondroid features are also frequently seen. Some tumors exhibit minimal or no cystic component, and these lesions have been designated as "solid variant of ABC" and "giant cell reparative granuloma". The latter term is particularly used for lesions that occur in craniofacial bones and the small bones of the hand and feet.[1,8,19,22–25]

The pathogenesis of ABC has been a subject of debate since its original description.[1] However, identification of recurrent chromosomal translocation t(16;17)(q22; p13) by Panoutsakopoulos et al[9] strongly indicated a neoplastic pathogenesis in at least a subset of primary ABC. These findings were confirmed by additional cytogenetic reports,[10–15] which also showed similar cytogenetic abnormalities in the solid variant of ABC and in soft tissue ABC.[10,26] Furthermore, these studies showed that chromosome 17p13 is the most frequently rearranged chromosomal region in ABC.

Recently, we demonstrated that the chromosomal translocation t(16;17)(q22;p13) fuses the promoter region of the osteoblast cadherin 11 gene (*CDH11*) on chromosome 16q22 to the entire coding sequence of the ubiquitin protease *USP6* gene on chromosome 17p13.[16] We also found that *CDH11* and *USP6* loci were rearranged in ABCs with alternate translocations involving either chromosome 16q22 or 17p13. These studies suggested that the pathogenesis of some primary ABC involves transcriptional up-regulation of *USP6*. The transforming mechanisms of *USP6* remain to be determined but could involve known *USP6* roles in regulation of actin remodeling through interactions with the Rho GTPases Cdc42 and Rac1.[27]

In the present studies, we demonstrate *CDH11* or *USP6* rearrangements in 69% of primary ABC. Notably, 44% (23 of 52) had variant *USP6* rearrangements, in the absence of *CDH11* rearrangement. These findings suggest that *USP6* could be the most prevalent fusion partner in ABC, with variant translocations providing alternative oncogenic mechanisms for *USP6* transcriptional up-regulation. The identification of three cases in which only the *CDH11* locus was rearranged, without evidence of *USP6* rearrangement, also suggests that genes other than *USP6* are up-regulated by juxtaposition with *CDH11* in ABC.

The possibility of a genetic relationship between primary and secondary ABC was suggested recently by the finding of chromosome band 16q22 and 17p13 rearrangements in a giant cell tumor with secondary ABC.[21] Nonetheless, in contrast to the situation in primary ABC, we found no evidence of *CDH11* or *USP6* rearrange-

ments in secondary ABC associated with giant cell tumor, chondroblastoma, osteoblastoma, and fibrous dysplasia. Although chromosome 16 and 17 abnormalities have been found infrequently in giant cell tumors,[21,28,29] it is unclear whether any of these involve *CDH11* or *USP6* loci. Based on the present evidence, we conclude that secondary ABC and primary ABC have a different pathogenesis. Secondary ABC, presumably, is a common endpoint of differentiation or a non-specific morphological pattern in various non-ABC tumors. Despite being a morphological mimic of primary ABC, secondary ABC likely has varied genetic features, corresponding to the specific bone tumors with which it is associated.

Presence of *USP6* and *CDH11* rearrangements did not correlate with ABC clinicopathological variables, nor, in univariate and multivariate analyses, with recurrence-free survival. The only variable that correlated with recurrence-free survival was tumor location, with central ABC being at greater risk for recurrence. However, we cannot exclude the possibility that specific *USP6* or *CDH11* rearrangement mechanisms are predictive of recurrence. Therefore, it would be worthwhile to evaluate relationships between molecular aberrations and ABC recurrence further, ideally in the context of a larger-scale and prospective clinical study.

The paraffin-section FISH analyses in this study enabled evaluation of ABC cell types which had the *USP6* and *CDH11* rearrangements. All cells with *USP6* or *CDH11* rearrangements were spindle or ovoid, and were diffusely admixed within the other ABC cell components, as well as being clustered around osteoclast-like multinucleated giant cells. The lack of cytogenetic aberrations in the inflammatory cells, endothelial cells, metaplastic bone-associated osteoblasts, and the multinucleated osteoclast-like giant cells supports their reactive nature. Conceivably, they are involved in a host response to the neoplastic ABC cells. It is also possible that one or more of the non-neoplastic cellular constituents release factors that stimulate division of the neoplastic ABC cells. The frequent clustering of neoplastic cells in the vicinity of multinucleated giant cells suggests a role for these cells in the neoplastic proliferation. Notably, the ABC cells with *USP6* or *CDH11* rearrangements were generally admixed with morphologically indistinguishable spindle cells lacking such rearrangements. We hypothesize that the latter group is composed by reactive fibroblasts/myofibroblasts, but it is also possible that some of these are committed ABC neoplastic cells at an earlier point in transformation, before acquisition of the *USP6* or *CDH11* rearrangements. Although the majority of cells in some ABC had *USP6* or *CDH11* rearrangements, there were also tumors in which fewer than 10% of cells had rearrangements (Table 2). The small numbers of apparently rearranged cells were not false-positive findings, as they were reproducibly present in these tumors when the FISH was repeated, and when scored by independent observers. Furthermore, RT-PCR reactions corroborated these molecular cytogenetic results showing the presence of *CDH11-USP6* fusion transcripts in these tumors. In all, these findings suggest that the neoplastic ABC component induces a vigorous, reactive, host response mimick-

ing young granulation tissue, including inflammatory, myofibroblastic, osteoclast-like giant cells, osteoblastic cells, and numeous capillaries, therefore accounting for the historical perception that these are largely reactive lesions.

In conclusion, primary ABC is a mesenchymal neoplastic disease characterized by a spindle cell proliferation exhibiting *USP6* or *CDH11* rearrangements in approximately two thirds of the cases. Secondary ABC is a morphological mimic of primary ABC, but lacks the hallmark *USP6* or *CDH11* rearrangements of primary ABC, and likely represents a common endpoint of differentiation in various non-ABC bone tumors.

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