Clonal Rearrangement of Chromosome Band 6p21 in the Mesenchymal Component of Pulmonary Chondroid Hamartoma

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ABSTRACT

Pulmonary chondroid hamartomas (PCH) are biphasic benign tumors that contain both mesenchymal and epithelial populations. In this report we describe two PCH in which clonal translocations at chromosome band 6p21 were demonstrated in mesenchymal cells. One of these had a unique translocation, t(6;14)(p21;q24), that was also found in one of two PCH karyotyped previously. The t(6;14) has not been described in other varieties of benign or malignant neoplasia. The 6p21 aberrations are of particular interest because break points in this chromosomal region appear to be characteristic of endometrial polyps. Endometrial polyps, like PCH, are biphasic benign tumors in which mesenchymal clonality has been demonstrated.

INTRODUCTION

PCH are unusual benign tumors that occur in both children and adults. PCH are generally asymptomatic nodules that arise in the periphery of the lungs, and most cases are discovered incidentally during routine radiographic studies or at postmortem examination (1, 2). Most PCH are composed of epithelial-lined clefts that are situated within a heterogeneous proliferation of mesenchymal cells. The mesenchymal population in a given PCH might include fibrous connective tissue, fat, and mature or/and immature chondroid tissue. Although PCH were thought originally to represent hyperplastic developmental remnants, it has been suggested that the epithelial and mesenchymal components, or the mesenchymal component alone, might be neoplastic (1–4). Malignant transformation in PCH is exceptionally rare, however, and cure is accomplished invariably by surgical resection (5).

We have described clonal chromosome rearrangements in the mesenchymal components of two PCH (6). One of those tumors contained a balanced translocation (6;14)(p21;q24) and a chromosome 11 long arm deletion, whereas the other contained clonal rearrangements of chromosomes 12 and 18. Herein, we report two additional cases of PCH in which clonal rearrangement at chromosome band 6p21 was demonstrated in mesenchymal cells.

MATERIALS AND METHODS

Case Reports

Case 1. A 37-year-old woman underwent wedge resection of an asymptomatic right lower lobe PCH that was 0.8 cm in its greatest diameter. Histologically, the PCH was composed of varied mesenchymal cell types along with lumina lined by benign columnar or cuboidal epithelium (Fig. 1). The mesenchymal components included immature cells within a myxoid stroma (65% of the total mesenchyme), mature adipose tissue (20% of the total mesenchyme), and mature cartilage (15% of the total mesenchyme).

Case 2. A 68-year-old man underwent wedge resection of an asymptomatic left lower lobe PCH that was 2.5 cm in greatest diameter. Histologically, the PCH was composed predominantly of mesenchymal cell populations that included immature cells in a loose, myxoid stroma (80%), scattered foci of adipose tissue (15%), and a minor component of mature cartilage (5%). The PCH also contained scattered lumina, lined by cuboidal or columnar epithelium, that in some cases were associated with chronic inflammatory cells. The epithelial cells were noted primarily at the periphery of the hamartoma.

RESULTS

Cytogenetic Analyses

Sterile specimens of each PCH were obtained directly from the frozen section room and were disaggregated, cultured, and karyotyped using methods described previously (6). Metaphase cells were harvested after 5–6 days in culture. Chromosome abnormalities were described using the conventions proposed in the International System for Human Cytogenetic Nomenclature (1991) (7). Fluorescent in situ hybridization studies were carried out with a spectrum orange-labeled probe cocktail to chromosome 6 (Imagenetics, Naperville, IL) and a biotinylated probe cocktail to chromosome 14 (Oncor, Gaithersburg, MD), according to the manufacturers’ protocols.

Immunohistochemical/Cytogenetic Analyses

Combined immunohistochemical/cytogenetic analyses were carried out on fresh metaphase cell preparations as described previously (6). The immunohistochemical/cytogenetic analyses used APAAP immunohistochemical staining to determine tissue lineages of quinacrine-banded metaphase cells. Mesenchymal and epithelial cell lineages were established using mouse monoclonal antibodies to vimentin (Dako Corp., Carpinteria, CA) and keratin proteins (AE1/AE3; Boehringer Mannheim, Indianapolis, IN), respectively. Incubations with the primary antibodies were followed by incubations with rabbit anti-mouse immunoglobulin antibodies and APAAP complexes (Dako). APAAP complexes were detected using naphthol AS-MX phosphate (Sigma Chemical Co., St. Louis, MO) as substrate and Fast Red TR salt (Sigma) as chromagen.

Cytogenetic Analyses

Case 1. Phase microscopy at the time of metaphase cell harvests revealed spindled (mesenchymal) and epithelial morphologies in 80 and 20%, respectively, of cultured cells. Twenty metaphase cells were analyzed after trypsin-Giemsa banding. Thirteen cells were pseudodiploid: 46,XX,der(6)(6;14)(p21;q24)-;ins(6;14)(p21q22q23),(7;15)(q22q22),der(14)(6;14)(p21;q24) (Fig. 2), and the remaining seven cells were diploid: 46,XX. The translocation break points in the t(6;14) were identical to those in a PCH that we reported previously (Ref. 6, case 2). However,
REARRANGEMENT OF 6p21 IN PULMONARY HAMARTOMA

Fig. 1. Histological section of pulmonary chondroid hamartoma, case 1, demonstrating epithelial and mesenchymal components, including mature cartilage, adipose tissue, and immature cells in a myxoid stroma. H & E; original magnification, x 100.

the derivative chromosome 6 in the present case also appeared to contain a chromosome 14 insertion near the translocation site.

Case 2. At time of metaphase cell harvests, all cultured cells had spindled (mesenchymal) morphology. Twelve metaphase cells were analyzed after trypsin-Giemsa banding. All cells were pseudodiploid with identical karyotypes: 46,XX,t(6;10)-(p21;q22) (Fig. 3).

In Situ Hybridization Analyses

The t(6;14)(p21;q24) in PCH case 1 and in one earlier case (Ref. 6, Case 2) were evaluated using fluorescent in situ hybridization with the chromosome 6 and chromosome 14 probe cocktails. Translocation of chromosome 6 and chromosome 14 material was seen in most metaphase cells from both cases (Fig. 4). These findings supported the presence of a reciprocal t(6;14) in both hamartomas.

Immunohistochemical/Cytogenetic Analyses

The tissue lineages of pseudodiploid and diploid metaphase cells were assessed in PCH case 1. Metaphase cell lineage was not evaluated further in case 2 because all cultured cells from that case had a mesenchymal morphology.

Vimentin staining was assessed in 29 quinacrine-banded metaphase cells from case 1. Vimentin was apparent in 26 cells, 19 of which were pseudodiploid (Fig. 5) whereas 7 were diploid. Three cells lacked vimentin, and each of these was diploid.

Keratin staining was assessed in 12 metaphase cells from case 1. Keratin was detected in only four metaphase cells, all of which were diploid. Of the eight cells that lacked keratin staining, five were pseudodiploid (Fig. 5) and three were diploid.

Histological Comparison of Four Cytogenetically Abnormal PCH

Histological comparisons were made for the present two PCH, the previously reported PCH with t(6;14) (6), and the previously reported PCH which contained clonal chromosome 14 homologue (Ref. 6, Case 2).
rearrangements but lacked t(6;14) (6). This assessment was carried out by one of us (J. L.) who was blinded as to which cases contained the chromosome band 6p21 translocations. All four PCH contained some lumina lined by benign columnar or cuboidal epithelium. The previously reported PCH with t(6;14) was composed predominantly of immature mesenchymal cells in a loose myxoid stroma; this histology was similar to that in the present two cases. By contrast, the PCH which lacked chromosome band 6p21 rearrangement was composed predominantly of mature cartilage nodules with only minor components of primitive mesenchymal stroma. These preliminary findings suggest that chromosome band 6p21 rearrangement might be associated with primitive mesenchymal proliferation in PCH.
REARRANGEMENT OF 6p21 IN PULMONARY HAMARTOMA

Fig. 5. Vimentin (A, B) and keratin (C) immunohistochemical staining of clonal quinacrine-banded metaphase cells from pulmonary chondroid hamartoma case 1. Short arrows indicate the chromosome 6 homologues; long arrows indicate the chromosome 7 homologues. A, intense vimentin staining. Top, abnormal chromosome 7; bottom, abnormal chromosome 6. B, moderate vimentin staining. Abnormal chromosome 6 and abnormal chromosome 7 are in the right-hand aspect of this metaphase cell. C, negative keratin staining. Abnormal chromosomes 6 and 7 are the lowermost chromosomes in this cell.

DISCUSSION

In this report, we have described translocations at chromosome band 6p21 in the mesenchymal component of two PCH. These findings support histological indications that PCH represent neoplastic mesenchymal proliferations (2). The present findings, together with those we reported earlier (6), indicate that rearrangement of chromosome band 6p21 is a nonrandom cytogenetic aberration in PCH. Two of the cases with 6p21 aberrations had a translocation, t(6;14)(p21;q24), that has not been reported in other neoplasms (8) and might, therefore, be specific for PCH. This translocation was confirmed using fluorescent in situ metaphase hybridization with chromosome 6 and chromosome 14 libraries. Benign pulmonary chondroid neoplasms are also seen in the Carney's triad of gastric epithelioid leiomyosarcoma, extraadrenal paraganglioma, and pulmonary chondroma that has been described in young female patients (9). Predisposition to the component neoplasms in Carney's triad has not been linked, however, to 6p21.

It is intriguing that three PCH with translocations at 6p21 were composed primarily of immature mesenchyme, whereas one case that lacked 6p21 rearrangement was composed primarily of mature cartilage. These findings suggest the presence of a differentiation-associated genetic aberration in PCH. Cytogenetic analyses of additional PCH will be required to determine the validity of this potential association.

It is also intriguing that two other benign neoplasms, endometrial polyps and benign pleomorphic adenomas of the salivary gland, have characteristic translocation break points at 6p21 (10–12). Endometrial polyps, like PCH, appear to contain reactive epithelial cell populations and clonal mesenchymal populations (11). Pleomorphic salivary gland adenomas contain admixtures of mesenchymal and epithelial cells, but the clonal relationship of these cell types has not been determined. These findings suggest that benign mesenchymal proliferations with clonal 6p21 aberrations might be particularly likely to encourage reactive proliferation of adjacent epithelial cells.

Several oncogenes and growth factor genes map to 6p21. These genes include those for p33pim, cyclin D3, and tumor necrosis factors α and β (13–15). Tumor necrosis factor α can stimulate normal fibroblast growth (16), and this factor has also been implicated in the development of bleomycin-related pulmonary fibrosis (17). It is unknown, however, whether the tumor necrosis factors, p33pim, or cyclin D3 promote growth of more primitive mesenchyme. Several other oncogenes, including FYN, ROS1, and MYB, map to the long arm of chromosome 6 (18) and are unlikely to be activated by the 6p21 translocation.

The combined immunohistochemical/cytogenetic approach has many potential applications in defining the clonal relationships of different cell populations in multiphasic solid tumors (6). This approach is particularly efficient in mixed mesenchymal and epithelial neoplasms because vimentin and keratin are often specific markers, respectively, for the mesenchymal and epithelial components of these tumors. The present study confirms our previous reports (6, 11) that antibodies to vimentin and keratin react well with metaphase cells fixed conventionally in methanol/acetic acid (3:1).

Interactions between mesenchymal and epithelial cells appear to be important in embryogenesis and in postnatal tissue development and repair (19–20), and these interactions might occur in biphasic mesenchymal-epithelial neoplasms. Normal and neoplastic mesenchymal cells can stimulate the growth of epithelial cells by a variety of mechanisms (20–23); fibroblasts, for example, can release soluble factors that promote epithelial differentiation and proliferation (22–24). Release of such factors by a neoplastic mesenchymal population might have promoted reactive epithelial proliferation in the present cases of PCH. Isolation and characterization of any such factors might contribute substantially to our understanding of tissue development, tissue repair, and oncogenesis.
REFERENCES

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