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Cytogenetic and CGH studies in a case of pulmonary hamartoma

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Hamartomas are the most frequent benign tumor of lung that occur in adults and occasionally in children. They produce no symptoms but are often discovered incidentally on chest X-rays. In this report we describe a case of hamartoma. Cytogenetic studies were performed on short term tissue culture from the histopathologically confirmed hamartoma mass. A clonal chromosomal aberration add (6) (p21) was observed. Comparative genomic hybridization (CGH) showed both gains and losses on different chromosomes in detail in whole genome, and also confirmed former cytogenetic findings in this patient. [Turk J Cancer 2001;31(1):15-20]

Key words: Hamartoma, cytogenetics, CGH

Hamartomas designate an excessive focal overgrowth of mature normal cells and tissues in an organ, composed of identical cellular elements. They are encountered in various parts of the body. Hamartomas are the most common benign tumors, majority of which are asymptomatic nodules diagnosed by chance (1,2). Here, we report both cytogenetic findings and gains and losses of DNA sequences in a case of hamartoma by CGH using modified double step DOP-PCR allowing amplification and labelling of whole genome.

Case Report

A thirty-eight year old woman, presented with left lateral back-ache, night fever and cough. Chest X-ray revealed a well-demarcated round lesion in the left upper lung. Thoracotomy was performed and the mass was removed from the left upper lung. The extracted specimen, measuring 3 x 2,5 x 2 cm was fragile in consistency. The cut surface showed gray whitish nodular pattern with some hemorrhagic areas. Histopathological picture showed lobules of cartilage. The lobules were separated by clefts and tubules, lined by pseudostratified

respiratory epithelium. Based on these findings, the diagnosis of hamartoma was made (Figure 1).

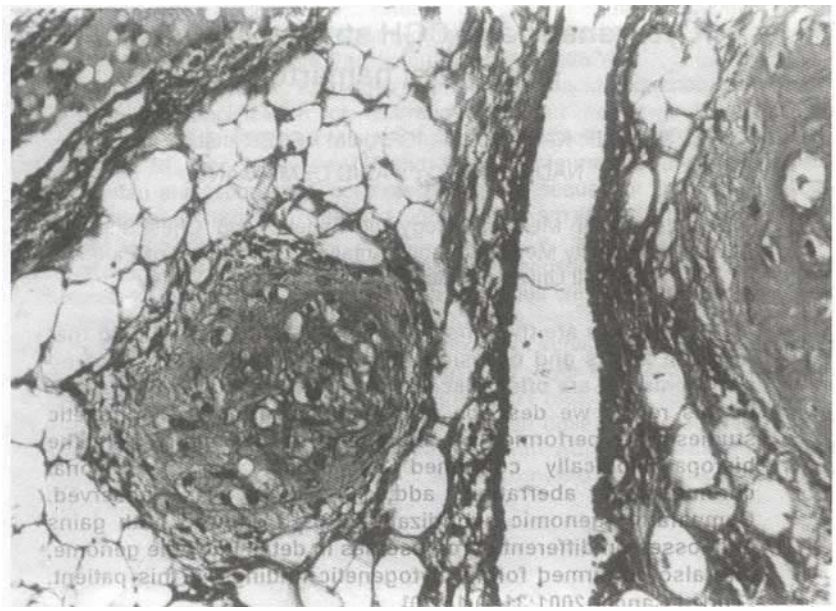

Fig 1. Microphotograph of the lesion of showing lobules of cartilage separated by clefts lined by respiratory epithelium

Short-term cultures were initiated from fresh tissue in RPMI 1640 (Irvine Scientific) with HEPES buffer supplemented with foetal calf serum (10%) (Irvine Scientific), L-Glutamine (Irvine Scientific), penicilline-streptomycin (Irvine Scientific), epidermal growth factor (Sigma) and insulin (Sigma). After 3-7 days, the cultures were exposed to colcemid (0,01 mg/ml) (Gibco) for 4 hours and harvested by hypotonic treatment in 0,06 M KCL (Merck) and repeated fixations in methanol:acetic acid (3:1). The slides were incubated at 37^{o}C for 3 days and then G-banded with Giemsa (Merck) stain. Thirteen out of the twenty-five metaphases analysed showed the following karyotype 46,XX, add (6) (p21) (Figure 2). The remaining cells were normal. Also nonclonal abnormalities were found as del (3) (q21q29), del (7) (q32q36).

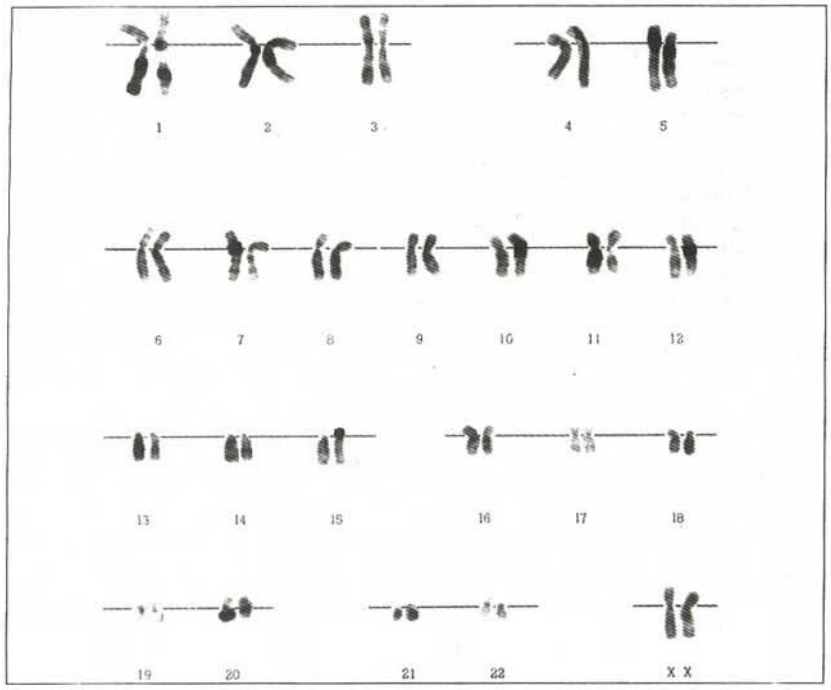

Fig 2. Karyotype of the patient

Table 1
Comparative genomic hybridization results of a case with hamartoma

LOSSES	GAINS
4q28-qter	1p33-pter
6p22-pter	4p15-p16
9pter-q13	5p
13p31-q33	5q14-q22
	5q23-qter
	6p21.3
	11
	14q31-q32
	18q12-q22
	19q
	20p13-qter
	21q22
	22
	Xp21

Test DNA was obtained from paraffin-embedded tumor sample scraped from hamartoma cell areas with sterile scalpel using modified single step DOP-PCR (Degenerate Oligonucleotid Primed-Polymerase Chain Reaction) (3-5). This first-step DOP-PCR amplified DNA of test and normal human (female) DNA were labeled with Spectrum Green-dUTP and Spectrum Red-dUTP (Vysis Co.) respectively by second-step DOP-PCR. Hybridization was performed as described earlier (6). Briefly, test DNA and normal human DNA were hybridized to slides of metaphase cells from the blood of a healthy donor (female). Following hybridization for 2 days, slides were washed, and chromosomes were counterstained with DAPI (4,6-diamino-2-phenylindole, 200 ng/ml). Digital image analysis; using PSI (Perceptive Scientific International, LTD) Workstation image processing and evaluation were described in detail before (7). Losses and gains of chromosomal regions in this patient obtained as an outcome of CGH (Figure 3) are shown in Table 1.

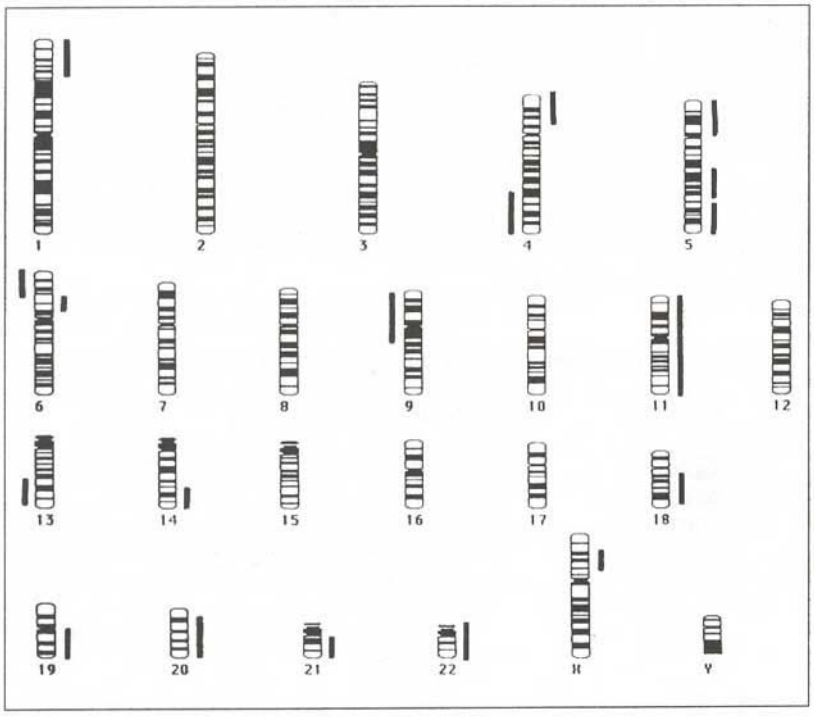

Fig 3. Summary of all chromosomal gains and losses identified in a case of hamartoma. Vertical lines on the right side and left side of chromosome indicate a gain and loss of genetic material, respectively

Discussion

Somatic cell mutations are found in the solid tumors as clonal chromosomal anomalies (8). Hamartomas are benign tumors of which the clonal chromosome aberrations have been previously described in hamartoma of lung (9-13). The most characteristic aberrations identified in hamartomas involved chromosome band 6p21.

The chromosome band 6p21, locating high-mobility group gene (HMGI[Y]), encodes DNA binding proteins (14). Although HMGI[Y] expression is associated with neoplastic transformation, oncogenic HMGI[Y] mutations have not been identified (15). In this case, we have also found a derivative chromosome of 6, as the only clonal chromosomal abnormality. An additional material was attached to band 6p21, but neither the origin of the extra segment nor the type of rearrangement was known. Although the origin of the additional material was not known, this cytogenetic finding has supported that HMGI[Y] gene has an oncogenic role in the pathogenesis of pulmonary hamartoma. Then, CGH was performed, and whole chromosome gains, amplifications and deletions of different chromosomes were found on the short and long arms of chromosomes including chromosome 6p21. As a result, additional material at 6p21 may be originated from 6p21 locus itself or from other defined chromosomal amplification regions (Table 1). It is also important to mention that while only an additional region at 6p21 was observed cytogenetically in our study, CGH revealed other regions of gains and losses as well on various chromosomes, that cytogenetic methods failed to detect.

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