Author's response to reviews

Title: Hidden chromosomal abnormalities in Pleuropulmonary Blastomas identified by Multiplex FISH

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Author's response to reviews: see over
COVERING LETTER

To the Editor:
referee 2 proposes the manuscript could be shortened without losing the message but referee 1 requires described methodologies with detailed FISH results following the ISCN nomenclature. We propose this modified version to the both referees.

REFEEEEREEEREE 1

1.1) Chromosome analysis was performed on tumor fragments obtained from the primary (case2) or recurrent tumors (case1 and 2). For culture, fragments of sterile tumor tissue were dissociated enzymatically using an RPMI medium containing 0.02% collagenase type II and incubated at 37°C for approximately 1 hour. A cell suspension obtained after centrifugation was seeded into 25 cm2 tissue culture Falcon flasks with RPMI medium supplemented with 10% fetal bovine serum and antibiotics. The cells were then incubated at 37°C in a 5% CO2 atmosphere. After culture entered exponential growth (10 days), chromosomal analysis was performed using a routine cytogenetic technique. R-banding technique was used for chromosomal identification.

1.2) Section II 2: pathology methods have been briefly described and pathological findings have been located in section III-1 RESULTS:

II 2 – PATHOLOGY

Tumor specimens were fixed in formalin and paraffin embedded for histologic and immunohistochemical analysis. Paraffin-embedded tissues were sectioned at 4 µm and stained with hematoxylin-phloxin-safron (HPS). For immunohistochemistry the sections were deparaffined and subjected to antigen retrieval. An automated immunohistochemistry was performed with avidin-biotin-peroxidase complex on a Ventana 320 device (Tucson, AZ, USA) with a Ventana kit (Strasbourg, France) including AEC reagent. The following primary antibodies (dilution and source in parenthesis) were used: glial fibrillary acidic protein (GFAP, Dakopatts, 1/2000), S-100 protein (Dakopatts, 1/400), α-smooth actin (Dakopatts, prediluted), desmin (Immunotech, prediluted), myogenin (Microm, 1/5).

1.3) and 1.4) Karyotype description and FISH abnormalities have been modified in section III-2 and in FIGURE LEGENDS following the ISCN nomenclature:

Case 1:
42, X, -X, der(8), der(11), -15, der(17), -18, del(20)(p12pter), -21 .ish der(8)t(8;18)(D8Z1+,wcp8+,wcp18+) .ish der(11)dic(11;21)(wcp11+,wcp21+) .ish der(17)t(15;17)(D17Z1+,wcp15+,wcp17+,TP53-)

Case 2:
81, XXXYY, +der(1)x2, +2, +2, +der(3)x2, +4, +4, +der(?4), +5, +6, +6, +7, +der(7), +8, +8, +8, +der(9), +11, +12, +12, +14, +16, +18, +18, +der(19)x2, +20, +20, +21, +22, +22
1.5) The percentage was 32%

2.1) For reference 23, we suggest a lead article: Sandberg et al. “Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: lipoma” - 2004
For reference 24, we suggest an article: Martins et al. “PLAG1 gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: a combined study using chromosome banding, in situ hybridization and immunocytochemistry” - 2005
Reference 30 has been modified.

Reference 27 has been modified.
This data has been reported in reference 25 too (Steenman et al.)

2.3) “and al” was modified by “et al.” in text and in Table I

3) Indeed, Spencer et al described classic ADULT type pulmonary blastoma. In 1988, Manivel et al. described PPB in CHILDREN as a distinctive intrathoracic/pulmonary neoplasm.
Correction has been made in text.
We precise cytogenetic datas in our both cases too (see section I)

4) Paragraph 2 from Section II-2 has been modified and has been included in section III-1: III1-Pathological findings

On microscopic examination, both tumors were made of solid sheets of immature cells (figures 1, 2a), some being multinucleated (figure 2c). Mitoses were numerous and necrosis was also present. No cystic area was noted. In case n°1, immunohistochemistry was negative for GFAP and S100 protein that ruled out a malignant glioma. Myogenic markers were also negative. In case n°2, at the first surgery, a malignant mesenchymal component showing multidirectional differentiation was associated with the blastema. Areas containing immature cartilage and rhabdomyoblastic cells were present. The myogenic differentiation was confirmed by immunohistochemistry. Some cells were positive for desmin and myogenin (figure 2b, 2c). At recurrence case n°2 only showed areas of poorly differentiated blastematous cells (figure 2d). Pathological diagnosis in both cases was PPB type III.
5) Yes, we agree with you. So, we have been used the term “likely”.

6) Correction is: 11(p11pter)

7) Correction has been made in text

8) As suggested, we decide to modify paragraph (see text)
Bonner et al. demonstrated that transcriptome wide analysis of gene expression by DNA array technology showed numerous patterns associated with lung development in murines. The authors concluded that the genes identified as relevant to lung development and belonging to three regulatory pathways (Wnt/β-catenin signalling, cell cycle and apoptosis) may be suggested as candidate susceptibility genes for lung tumorigenesis. Chromosome 1p alterations harboring developmental genes related to the Notch and Wnt pathways have been reported also by Garnis et al. Further similar studies in embryonal tumors could be proposed to contribute to our understanding of unusual characteristics in the PPB development.

9) Correction have been made for figure 2, 3 and 4.

REFEREE 2

1) Karyotypes and FISH results have been revised following the ISCN nomenclature (see text and figure legends)

2) As suggested, the “doubled population” at recurrence has been added in text.

Due to first reviewer’s report (pathology and cytogenetic methodologies to detail and discussion to precise), it was difficult to write a shorter version. If necessary, we could.