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Original contribution

Thyroid paraganglioma. Report of 3 cases and description of an immunohistochemical profile useful in the differential diagnosis with medullary thyroid carcinoma, based on complementary DNA array results☆

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Summary Thyroid paraganglioma is a rare disorder that sometimes poses problems in differential diagnosis with medullary thyroid carcinoma. So far, differential diagnosis is solved with the help of some markers that are frequently expressed in medullary thyroid carcinoma (thyroid transcription factor 1, calcitonin, and carcinoembryonic antigen). However, some of these markers are not absolutely specific of medullary thyroid carcinoma and may be expressed in other tumors. Here we report 3 new cases of thyroid paraganglioma and describe our strategy to design a diagnostic immunohistochemical battery. First, we performed a comparative analysis of the expression profile of head and neck paragangliomas and medullary thyroid carcinoma, obtained after complementary DNA array analysis of

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2 series of fresh-frozen samples of paragangliomas and medullary thyroid carcinoma, respectively. Seven biomarkers showing differential expression were selected (nicotinamide adenine dinucleotide dehydrogenase 1 alpha subcomplex, 4-like 2, NDUFA4L2; cytochrome *c* oxidase subunit IV isoform 2; vesicular monoamine transporter 2; calcitonin gene-related protein/calcitonin; carcinoembryonic antigen; and thyroid transcription factor 1) for immunohistochemical analysis. Two tissue microarrays were constructed from 2 different series of paraffin-embedded samples of paragangliomas and medullary thyroid carcinoma. We provide a classifying rule for differential diagnosis that combines negativity or low staining for calcitonin gene-related protein (histologic score, <10) or calcitonin (histologic score, <50) together with positivity of any of NADH dehydrogenase 1 alpha subcomplex, 4-like 2; cytochrome *c* oxidase subunit IV isoform 2; or vesicular monoamine transporter 2 to predict paragangliomas, showing a prediction error of 0%. Finally, the immunohistochemical battery was checked in paraffin-embedded blocks from 4 examples of thyroid paraganglioma (1 previously reported case and 3 new cases), showing also a prediction error of 0%. Our results suggest that the comparative expression profile, obtained by complementary DNA arrays, seems to be a good tool to design immunohistochemical batteries used in differential diagnosis.

1. Introduction

Thyroid paraganglioma (TP) is an uncommon type of tumor, which may microscopically mimic medullary thyroid carcinoma (MTC). Several years ago, one of the authors (X. M.) reported a series of 3 cases and reviewed the features of 11 previously described TPs [1]. Because of the occasional description of positive immunoreactivity of cytokeratins and calcitonin in TP and also the presence of S-100–positive sustentacular cells in MTC, the authors suggested that a battery of epithelial, neural, and hormonal immunohistochemical markers were mandatory in differential diagnosis. Several additional examples of TP have been reported since then [2-15], and new markers have been also reported to be useful in this differential diagnosis.

In the present article, we describe the pathologic features of 3 new examples of TP. We have also tried to find the ideal immunohistochemical battery in the differential diagnosis between TP and MTC, after selecting a group of 7 proteins, corresponding to 6 transcripts that are differentially expressed in complementary DNA (cDNA) comparative expression analysis, between head and neck paragangliomas (PGL) and MTC. cDNA array results were validated in 2 tissue microarrays of 2 series of PGL and MTCs. Finally, the reliability of the immunohistochemical battery was tested with 4 examples of TP: the 3 new cases and 1 recently reported [4].

2. Materials and methods

2.1. Tissue specimens for cDNA analysis

Fifty-seven frozen tumors, 9 PGL and 48 MTC, were collected from different Spanish hospitals through the Spanish National Tumor Bank Network and from the Instituto Oncologico Veneto in Italy. The tissues were immediately frozen in liquid nitrogen, embedded in Tissue-Tek OCT compound (Sakura, Torrance, CA), and stored at −80°C until they were used. All tissues were evaluated by pathologists using hematoxylin/eosin staining; 85% was considered a suitable percentage of tumor cells per sample to be included in the study. All patients provided informed consent.

2.2. RNA isolation

Total RNA was obtained using the TriReagent kit (MRC, Cincinnati, OH), according to the manufacturers' instructions. Purity and integrity of RNA were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). All samples had an RNA integrity number above 7. Concentration was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.3. cDNA synthesis, labeling, hybridization, and detection

For each tumor, 500 ng of total RNA was amplified by double-strand cDNA synthesis, followed by T7-based in vitro transcription, according to the manufacturer's instructions. Universal Human Reference RNA (Stratagene, La Jolla, CA) was used for all samples as a reference. Amplified cRNA was then labeled with cyanine (Cy)5–conjugated 2′-deoxyuridine 5′-triphosphate, whereas cRNA from Universal Human Reference RNA was labeled with Cy3-conjugated 2′-deoxyuridine 5′-triphosphate. The Agilent Whole Human Genome platform (4 × 44K) was used for competitive hybridization, and the slides were washed, dried, and scanned in an Agilent microarray scanner (Agilent Technologies, Palo Alto, CA, USA).

2.4. Normalization and preprocessing

Two-channel ratios (Cy5/Cy3) for each array spot were generated and quantified using Feature Extraction version