Predictive Factors for Malignant Pheochromocytoma: Analysis of 136 Patients

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Purpose: We evaluated the clinical characteristic, tumor feature and immunohistochemistry factors predicting malignant pheochromocytoma.

Materials and Methods: Between January 1999 and December 2008 we retrospectively reviewed the records of 136 patients with pheochromocytoma at Ruijin Hospital. We compared clinical characteristics (age, gender, symptoms and biochemical analysis), tumor features (site, weight and diameter) and the expression of 3 angiogenesis/metastasis related genes (VEGF, Cox-2 and MVD) by immunohistochemical analysis of benign vs malignant pheochromocytomas.

Results: Of the 136 patients 105 (77%) had benign and 31 (23%) had malignant pheochromocytoma. Malignant tumors were larger and heavier than benign tumors, and accompanied by higher plasma metanephrine secretion (each $p < 0.001$). Mean tumor catecholamine and preoperative 24-hour urinary metanephrine or normetanephrine were obviously higher in malignant than in benign tumors ($p < 0.001$). Also, 25 malignant tumors (81%) were immunopositive for VEGF while only 24 benign tumors (23%) showed this characteristic ($p < 0.001$). Microvessel density and the rate of positive staining for Cox-2 protein in malignant samples were higher than in benign samples ($p < 0.001$).

Conclusions: Several promising predictive parameters are currently available to distinguish benign from malignant pheochromocytoma. Large (5 cm or greater) or heavy (250 gm or greater) tumors, multifocal and extra-adrenal tumors, early onset postoperative hypertension and higher plasma or urine metadrenaline are high risk factors predictive of malignant pheochromocytoma. Also, expression of the 3 angiogenesis or metastasis related genes VEGF, Cox-2 and MVD helps determine the diagnosis of malignancy and suggests strict followup.

Key Words: adrenal glands; pheochromocytoma; diagnosis, differential; metanephrine; gene expression

Abbreviations and Acronyms

Cox-2 = cyclooxygenase-2
CSI = chemical shift imaging
CT = computerized tomography
FDG = fluorodeoxyglucose
MIBG = ^{131}I-metaiodobenzylguanidine
MN = metanephrine
MRI = magnetic resonance imaging
MVD = microvessel density
NMN = normetanephrine
PCC = pheochromocytoma
PET = position emission tomography
SDH = succinate dehydrogenase
VEGF = vascular endothelial growth factor

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PHEOCHROMOCYTOMA is a catecholamine secreting tumor derived from sympathochromaffin cells. These tumors generally arise from the adrenal medulla but may also develop from extra-adrenal tissue as extra-adrenal PCC or paraganglioma.[1] They are usually benign but may also present as or develop into malignancy, as documented by lymph node, bone or visceral metastasis at the first operation or at recurrence. The prevalence of malignancy is 5% to 26% of PCC cases.[2] Tumors may recur months or years after the initial operation.

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Identifying primary malignant PCC remains essential to establish optimal treatment and followup. However, there are no reliable histological or biochemical markers to distinguish benign from malignant tumors. The diagnosis of malignancy requires evidence of metastasis to the site where chromaffin cells do not exist. Early diagnosis of malignant PCC is difficult. Currently controversies still exist in the diagnosis, treatment and prognosis of this rare disease.[3]

Over the years many investigators have tried to find distinguishing criteria that may be used to discriminate benign PCC from malignancy, allowing clinicians to tailor appropriate therapy and followup to the individual. Many immunohistochemical markers, such as Ki-67, human telomerase reverse transcriptase expression and SDH, subunit B gene mutation, are used to predict PCC behavior, correlated with malignancies.[4–6]

Human VEGF and MVD are useful predictors of the invasiveness and vascularity of various types of carcinoma. Moreover, up-regulation of the Cox-2 gene as a rate limiting enzyme in prostanoid biosynthesis is associated with malignant tumor progression and prognosis. However, VEGF, MVD and Cox-2 expression in PCC, and their relationship with the prediction of malignancy were not clarified until recently.

To predict the malignant behavior of PCC more easily we retrospectively reviewed the medical records of 136 patients with PCC at our institution between January 1999 and December 2008. After this review all tumor blocks were examined for molecular markers, such as VEGF, MVD and Cox-2 expression, on immunohistochemistry. We then established a comprehensive predictive outline of malignant PCC encompassing clinical features and hormonal findings, followed by an investigation of the clinical usefulness of a sensitive panel of immunohistochemical markers to assess malignancy in PCC cases.

MATERIALS AND METHODS

Patient Population and Clinical Features

Included in this retrospective study were 136 patients with PCC treated at Ruijin Hospital between January 1999 and November 2008. In all patients the PCC diagnosis was confirmed before surgery by increased plasma and urinary catecholamine (adrenaline and norepinephrine) or their metabolites (metanephrine and occasionally vanillylmandelic acid) and by specific, unequivocal imaging findings. MRI, MIBG scan and PET/CT were the diagnostic procedures of choice in patients with suspicious lesions.

We excluded from study patients with a personal or family history of PCC, head and neck paraganglioma, multiple endocrine neoplasia type 2A or 2B, von Hippel-Lindau disease or neurofibromatosis type 1. Malignancy was defined as lymph node and distant metastasis at the initial intervention or recurrent PCC during followup. Extra-adrenal disease was defined as the initial discovered tumor at an extra-adrenal site. Metastasis develops to sites normally devoid of chromaffin tissue, excluding paragangliomas and carotid body tumors. In our study benign extra-adrenal PCC included 18 cases of paragangliomas and 2 of carotid body tumor. In the malignant PCC group there were 10 cases of paraganglioma and 4 of metastatic PCC, including a mediastinal tumor in 1, lymph node metastasis in 2 and bladder PCC in 1.

To identify possible correlates of malignancy or recurrence we analyzed certain clinical parameters in clinically malignant cases and compared findings with those in benign cases. Parameters included clinical characteristics (patient age, gender, symptoms and biochemical analysis), tumor features (site, weight and diameter) and persistent postoperative hypertension. We also analyzed immunohistochemical markers relevant to tumor development and angiogenesis, including VEGF, MVD and Cox-2.

Each patient was evaluated at regular intervals postoperatively. Routine examination, including blood pressure measurement, plasma or urine catecholamine (MN/NMN), MRI, MIBG and PET/CT, were done every 3 to 6 months for 5 years and every 6 months thereafter to follow patients and possibly identify recurrence. Sustained hypertension was diagnosed in patients with blood pressure 140/90 mm Hg or greater and in those on antihypertensive drugs.

Immunohistochemistry

We obtained paraffin blocks prepared for routine pathological examination of the tumors. They were cut into 7 μm sections, which were mounted on silane treated slides. Immunohistochemistry was performed with anti-VEGF, anti-CD34, anti-Cox-2 antibody (Sigma®) (1:1,000 dilution). The previously described protocol[5] included biotinylated secondary antibody and avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, California).

Positive VEGF,Cox-2 and CD34 staining was characterized by purple-brown granules located diffusely in the cell cytoplasm. The lack of any obvious purple-brown or brown-red pigmentation was considered a negative finding. Cytoplasmic VEGF and Cox-2 expression in cells was evaluated semiquantitatively, as previously described.[6] Under high power microscopy at 100× magnification 5 visual fields were randomly selected per section and 200 cells were counted in each high power field. Staining was scored as the percent of cells stained, including 0—less than 5%, 1—5% to 29%, 2—30% to 50% and 3—greater than 50%, and as intensity, including 0—no, 1—weak, 2—moderate and 3—strong staining. Positive or negative expression was determined according to the combination of these 2 variables. A total score of greater than 3 was considered positive and a total score of 3 or less was considered negative.

We evaluated MVD as the gold standard to assess tumor angiogenesis according to a previously described