

## FURTHER CONSIDERATION OF A MOUSE MODEL MALIGNANT PHEOCHROMOCYTOMA FOR DIAGNOSIS AND TREATMENT

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### ABSTRACT

Malignant pheochromocytomas, which account for 10% of all cases of pheochromocytoma, are an extremely troublesome disease for urologists and endocrinologists. It is difficult to examine malignant pheochromocytomas using conventional histopathological methods, and the condition carries a poor prognosis and no useful treatment has yet been established. We established a mouse model for metastatic malignant pheochromocytomas and tried to identify metastatic-related genes, which can provide useful information for the diagnosis and treatment of malignant pheochromocytomas for the purpose of developing a new diagnostic method for malignant pheochromocytomas.

Metastasis suppressor genes affect the spread of several cancers and, therefore, may provide promise as prognostic markers or therapeutic targets for malignant pheochromocytoma. We hypothesized that the downregulation of metastasis suppressor genes in malignant pheochromocytoma may play a role in malignant behavior. Using quantitative real-time PCR, expression of five genes (Metap2, Reck, S100a4, Timp2, and Timp3) was verified as significantly lower in liver tumors than in subcutaneous tumors. Downregulation of these genes has been previously associated with malignancy of pheochromocytomas. These findings indicate that different microenvironments can differentially affect the expression of metastasis-related genes in pheochromocytomas, and that overexpression or underexpression of these genes need not be present when the tumor cells are initially disseminated. This model is very useful, as this cell produces tyrosine hydroxylase and has more of the pheochromocytoma phenotype than the traditional pheochromocytoma lines. However, the establishment of malignant pheochromocytoma lines in humans will be necessary for *in vivo* research.

**Keywords:** Malignant Pheochromocytoma, Metastasis, Gene expression

### INTRODUCTION

Malignant pheochromocytomas, which account for 10% of all cases of pheochromocytoma, are an extremely troublesome disease for urologists and endocrinologists. It is difficult to examine malignant pheochromocytomas using conventional histopathological methods, and the condition carries a poor prognosis and no useful treatment has yet been established<sup>1</sup>. Here, for the purpose of developing a new diagnostic method for malignant pheochromocytomas, we established a mouse model for metastatic malignant pheochromocytomas and tried to identify metastatic-related genes, which can provide useful information for the diagnosis and treatment of malignant pheochromocytomas<sup>2,3</sup>.

### MATERIALS AND METHODS

#### Cell lines

Mouse pheochromocytoma cells (MPC, 4/30 PRR) were maintained in RPMI 1640 containing 10% heat-inactivated horse serum, 5% fetal bovine serum and antibiotics. MPC cells were grown in tissue culture dishes without collagen.

#### Animals and tumor model

About 6- to 10-week-old female athymic nude mice (NCR-nu) were purchased from Taconic (Germantown, NY) and treated in accordance with institutional guidelines. To generate tumors *in vivo*, un-anesthetized mice underwent tail vein and subcutaneous injection with  $10 \times 10^6$  MPC cells suspended in a volume of 100  $\mu$ L PBS (Sigma Chemicals Co., St. Louis, MO) using 1 mL tuberculin syringes (Becton Dickinson, Franklin Lakes, NJ) with 30-gauge hypodermic needles. Mice were observed 3 times weekly for evidence of tumor growth or adverse effects (*e.g.*, distress, ascites, bleeding, paralysis or excessive weight loss). In preliminary studies we used a different mouse strain (NMRI) to establish this model; however, nude mice (NCR-nu) were determined to be the optimal model as they reproducibly generated hepatic lesions. This might suggest that antitumor immune response plays an important role in controlling pheochromocytoma. This work, however, was beyond the scope of the present study. The mice were treated in accordance with the procedures approved and recommended by National

Institute of Child Health and Human Development, Animal Care and Use Committee, and NIH Animal Research Advisory Committee document entitled *Endpoints in Animal Study Proposal*.

The gene expression profiles of non-metastasizing tumors generated by injecting MPCs subcutaneously were compared to those of liver tumors generated by injecting the cells intravenously<sup>2,3</sup>. Both types of tumors were compared to the cultured parental cell line. Both were compared to the cultured parental cell line. Tumors in the liver have a route of spread, anatomical distribution, and growth environment similar to naturally metastasizing pheochromocytomas, while intravenous injection of cells bypasses the initial steps of metastasis occurring spontaneously from a primary tumor.

### RESULTS AND DISCUSSION

Eight genes were upregulated in liver tumors, 15 in subcutaneous tumors and seven in both compared to the cultured cells. Using quantitative real-time PCR, expression of five genes (Metap2, Reck, S100a4, Timp2, and Timp3) was verified as significantly lower in liver tumors than in subcutaneous tumors. Downregulation of these genes has been previously associated with malignancy of pheochromocytomas. These findings indicate that different microenvironments can differentially affect the expression of metastasis-related genes in pheochromocytomas, and that overexpression or underexpression of these genes need not be present when the tumor cells are initially disseminated. The hepatic localization of tumors formed by intravenously injected MPC cells and the tumors' gene expression profile resembling that of naturally occurring pheochromocytoma metastases support the use of this model to study pheochromocytoma metastasis.

Tumors in the liver have a route of spread, anatomical distribution, and growth environment similar to naturally metastasizing pheochromocytomas, while the intravenous injection of cells bypasses the initial steps of metastasis occurring spontaneously from a primary tumor. We considered that gene clusters, the gene expressions of which are suppressed in the metastatic lesion of this model, are responsible for the suppression of metastases. Thus, by screening them with macroarrays, we verified the suppression of

these genes using the real-time PCR method. As a result, the suppression of expression in three genes, Metap2, S100a4 and Timp2 in liver metastases, was confirmed. These genes may be very important for the diagnosis and treatment of malignant pheochromocytoma. Since our articles were published, we have further considered and discussed our results; however, we would also need to clarify why metastases are expressed only in livers and not in the lungs. The reason this cell is less likely to be engrafted in the lungs may lead to clues for the treatment of malignant pheochromocytoma. This model is very useful, as this cell produces tyrosine hydroxylase and has more of the pheochromocytoma phenotype than the traditional pheochromocytoma lines (e.g. PC-12)<sup>4</sup>. In a physiological and biochemical sense<sup>5,6</sup>, it will be effective to use this mouse model as a disease model for pheochromocytoma. In fact, we tried to monitor the blood pressure of this model using a tail cuff. Unfortunately, the data were unstable and we were not able to publicize them in an article or conference.

However, the establishment of malignant pheochromocytoma lines in humans will be necessary for in vivo research.

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