Erythropoietin Synthesis by Tumor Cells in a Case of Meningioma Associated With Erythrocytosis

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While secondary erythrocytosis is often associated with tumors arising from the kidney, other tumors have been described to originate in the liver, uterus, ovary, adrenal gland, and central nervous system, among which cerebellar hemangioblastomas are involved in most instances. Two cases of meningioma associated with erythrocytosis have already been reported. We observed a 59-year-old female patient who had developed a frontal meningioma associated with erythrocytosis. Before surgery, she had a significantly elevated total red blood cell volume with a normal plasma volume. Serum erythropoietin (Epo) dosage assessed by radioimmunoassay was within the normal range. The tumor was removed and the pathologic study found a meningotheliomatosi meningioma. Total RNA from the tumor was hybridized to a monkey cDNA Epo probe. A strong 1.6-kb messenger RNA (mRNA) signal was observed, which is the expected size of human Epo mRNA. In situ hybridization with the 35S-labeled Epo probe was performed on frozen tumor tissue sections. A significant hybridization was observed in all the tumor cells, whereas the stroma was negative. Therefore, in this meningioma associated with erythrocytosis, Epo was produced by the tumor cells themselves.

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RNA Extraction and Northern Blot Analysis

Frozen tissues from the tumor were crushed to a powder and homogenized in 4 mol/L guanidine thiocyanate. Total RNAs were extracted by the method of Chirgwin et al. After glyoxal denaturation, 20 μg of total RNA was electrophoresed and transferred to a nylon membrane, and the blotted RNAs were hybridized to the 35P-labeled Epo probe.

For positive control, RNA extracted from a well-characterized Epo-secreting renal cell cancer were hybridized in parallel with the Epo probe.

In Situ Hybridization

Five-micrometer thick sections of unfixed frozen tumor tissue were prepared. They were fixed in 4% formalin in phosphate-buffered saline (PBS; 0.1 mol/L, pH 7.4) for 5 minutes and dehydrated in graded alcohols without a permeation step. Except for slight modifications, the in situ hybridization procedure has been previously described. Briefly, tissue sections were hybridized under a sealed coverslip overnight at 45°C in 15 μL of a solution containing 40% deionized formamide; 4X standard saline citrate (SSC), pH 6.2; 0.02% Ficoll; 0.02% polyvinylpyrrolidone; 0.02% bovine serum albumin; 10% dextran sulfate; 2 mg/mL yeast tRNA (Sigma, St. Louis, MO); 400 μg/mL salmon sperm DNA (Sigma); 400 μg/mL herring sperm DNA (Sigma); 10 mmol/L dithiothreitol; and 0.2 ng/μL of the 35S-radiolabeled probe (specific activity, 2 × 106 cpm/μg) denatured at 100°C for 2 minutes. Slides were rinsed twice at 45°C with gentle agitation in 40% formamide-4X SSC for 15 minutes, followed by a rinse in 4X SSC at 60°C for 30 minutes, and then three times in 2X SSC at room temperature for 15 minutes. Sections were dehydrated in graded alcohols and dipped in Kodak NTB2 emulsion (Rochester, NY) for autoradiography. After 10 to 12 days of exposure, the slides were developed in Kodak D19, fixed in Kodak A44, and stained with hematoxylin and eosin.

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To assess the specificity of the in situ hybridization signal, control procedures were performed as follows: (1) negative control: treatment of meningioma tissue sections with 50 μg/mL ribonuclease A (type III; Sigma) in 2X SSC at 37°C for 30 minutes, then a rinse in 2X SSC for 15 minutes, and then hybridization with the Epo probe; (2) negative control: hybridization with a 35S-radiolabeled human renal renin probe used as a nonrelevant probe; (3) positive control: hybridization of sections from the Epo-secreting renal adenocarcinoma with the Epo probe.

**RESULTS**

**Pathologic Diagnosis**

The tumor cells were arranged in nests of cellular whorls without psammoma bodies. They had an abundant eosinophilic cytoplasm and presented neither nuclear atypia nor mitosis. The tumor stroma was well vascularized without inflammatory cells. This histopathologic pattern was consistent with the diagnosis of meningotheliomatous meningioma.

**Immunohistochemistry**

**Diagnostic immunohistochemistry.** Frozen meningioma tissue sections were fixed in 4% formalin in PBS for 5 minutes, rinsed three times in PBS, and incubated for 30 minutes with the following antibodies: monoclonal anti-vimentin (Dakopatts, Copenhagen, Denmark) diluted at 1/20, polyclonal anti-neuron-specific enolase (Dakopatts) at 1/100, prediluted polyclonal anti-protein S100 (Immunotech, Marseille, France), and monoclonal anti-cytokeratin (KL1; Immunotech) at 1/100. After a rinse in PBS, the antibodies were shown by using a peroxidase-antiperoxidase technique (Immunotech) for the polyclonal antibodies and a peroxidase-labeled antimouse antibody technique (Amer sham, Les Ulis, France) for the monoclonal antibodies.

**Epo immunohistochemistry.** Frozen meningioma and Epo-secreting renal adenocarcinoma tissue sections were fixed in cold acetone and rinsed in PBS. They were incubated for 30 minutes with a rabbit polyclonal antibody against human recombinant Epo raised in our laboratory diluted at 1/10, 1/100, or 1/500, and shown with a peroxidase-antiperoxidase technique.

**DISCUSSION**

This patient presented with a frontal meningioma associated with erythrocytosis. The erythrocytosis was assessed by the increase in RBC volume with a normal plasma volume. As usually described, resolution of this erythrocytosis occurred...
after surgical excision. The tumor had the classic histologic appearance of meningioma\textsuperscript{24} and a normal immunohistochemical pattern.\textsuperscript{25} This study demonstrates that, in this meningioma associated with erythrocytosis, Epo mRNA is produced by the tumor itself. Furthermore, the cellular localization of Epo synthesis is clearly in the tumor cells and not in the adjacent vessels.

The occurrence of erythrocytosis is very uncommon in cerebral meningiomas, with only two prior cases reported.\textsuperscript{9,17} This contrasts with cerebellar hemangioblastomas, in which erythrocytosis has been reported in up to 20\% of the cases.\textsuperscript{13} Hemangioblastomas have been shown to contain cells that stain positively for Epo using immunohistochemistry.\textsuperscript{26,27} Given these published data on the one hand and the very strong Epo mRNA signal observed in the meningioma on the other, immunohistochemistry was performed as suggested by Rich.\textsuperscript{28} The results of our attempts to localize Epo protein within tumor cells by immunohistochemistry remained negative. It is likely that in meningioma cells, Epo is being secreted at a very rapid rate and cannot be detected by a specific antibody.

In the two previous reports of meningiomas associated with erythrocytosis,\textsuperscript{9,17} the tumors had features similar to our case, such as supratentorial location and meningotheliomatous...
classification. However, despite the evidence of erythropoietic activity in the tumor extracts from the two cases, no direct demonstration of Epo synthesis was established. In the present report, the detection of Epo mRNA by Northern blot analysis of the RNAs extracted from the tumor and the labeling of the tumor cells evidenced by in situ hybridization with the Epo probe support the hypothesis of Epo synthesis by the tumor itself. Recently, Trimble et al.29 clearly showed that, in a case of hemangioblastoma associated with erythrocytosis, the tumor produced Epo mRNA. However, the cell type, ie, stromal or vascular cells, responsible for this synthesis was not determined. The investigators described an elevated Epo level in the cyst fluid, but could not detect any elevation of the Epo in the serum. Likewise, our patient's serum Epo level was within the normal range, but, as previously described,30 this does not exclude the diagnosis of secondary erythrocytosis. We previously demonstrated Epo mRNA production by the tumor cells themselves in renal cell carcinomas associated with erythrocytosis.22 The precise mechanism of Epo gene activation is not understood. Indeed, neither Epo gene rearrangement nor abnormal size of the tumor Epo mRNA were reported.22 Moreover, this “ectopic” Epo production in the kidney was due to tumor cells derived from proximal tubular cells, whereas we31 and others32 showed that, in the mouse kidney, peritubular cells were the physiologic site of Epo synthesis. This “ectopic” site of Epo secretion by malignant kidney cells might be due to a deregression of the Epo gene induced by the malignant transformation. In meningiomas, tumors are derived from meningotheial cells, the characteristic resident cell of the leptomeninges, which are neither physiologically nor ontogenically involved in Epo synthesis. Moreover, the large majority of meningiomas follow a benign clinical course and are permanently cured by surgical excision; consequently, they are not considered to be malignant tumors. Thus, abnormal Epo gene expression might occur in tumor cells regardless of whether the tumor is benign or malignant.

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REFERENCES


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