Cerebellar hemangioblastoma is a rare cause of secondary erythrocytosis. Although the erythrocytosis is a result of erythropoietin (Ep) stimulation, direct evidence of Ep synthesis by the tumor has been lacking. In an erythrocytotic patient with a cerebellar hemangioblastoma we found elevated levels of Ep in the tumor cyst fluid and for the first time demonstrated Ep mRNA in the tumor by Northern blotting. This finding confirms cerebellar hemangioblastoma as a site of ectopic Ep production.

Erythropoietin (Ep) is a glycoprotein hormone produced mainly by the kidney and is the principle regulator of erythropoiesis. The level of Ep is normally tightly controlled and helps to maintain a relatively constant red blood cell (RBC) mass. Occasionally, abnormal production of Ep results in secondary erythrocytosis. Secondary erythrocytosis has been reported in a variety of tumors including cerebellar hemangioblastoma, a rare intracranial neoplasm.

First recognized in 1943 by Carpenter et al., secondary erythrocytosis in cerebellar hemangioblastoma has been reported to occur in up to 20% of patients. Erythropoiesis-stimulating activity has been found within tumor cyst fluid, tumor extracts, cerebrospinal fluid, and, occasionally, serum. However, direct evidence that these tumors were actively synthesizing Ep was lacking. The recent cloning of the human Ep gene has provided molecular probes for the definitive identification of Ep-producing tissues. We report the presence of Ep in the tumor cyst fluid and the detection of Ep mRNA in the tumor from an erythrocytotic patient with a cerebellar hemangioblastoma. This finding confirms the tumor as the site of the ectopic Ep production.

CASE REPORT

A 40-year-old white man was admitted with a suspected cerebellar neoplasm. He presented with a 2-month history of headache, nausea, vomiting, and unsteadiness. Past medical and family histories were unremarkable. On examination he had bilateral papilloedema with disc haemorrhages, dysmetria of the right hand, decreased vision, and decreased tandem gait. An ophthalmologist found an enlarged blind spot and no evidence of retinal angioma. His initial hemoglobin was 18 g/L, hematocrit 54, RBC count 6.2 × 10¹²/L. The leukocyte and platelet counts were normal. A serum ferritin, serum B12, leukocyte alkaline phosphatase, baseline coagulation, and serum iron saturation were normal. A computerized tomography (CT) scan of the brain showed obstructive hydrocephalus due to a midline cerebellar tumor. The mass was predominantly cystic with a nodular component to the right of midline. The lesion enhanced with contrast. Angiography demonstrated a vascular cerebellar tumor supplied predominantly by the posterior-inferior cerebellar artery. The radiologic appearance was consistent with a cerebellar hemangioblastoma.

On the second hospital day the hydrocephalus was relieved by placement of a ventriculo-peritoneal shunt. Nine days later the tumor was completely excised. The patient had an uneventful postoperative course. At follow-up 9 months later he remains clinically well and the CT scan shows no recurrence. The hemoglobin returned to normal after removal of the tumor and has remained normal.

Histologic examination of the tumor showed numerous small capillaries and occasional larger vessels with intervening small groups of stromal cells. There was moderate nuclear pleomorphism but no mitoses. These features are those of a cerebellar hemangioblastoma.

MATERIALS AND METHODS

Measurement of Ep concentration. Preoperative and postoperative serum samples as well as the cyst fluid drained during excision were frozen at −70°C until assayed. Ep determinations were performed by a specific and sensitive radioimmunoassay using radiolabeled recombinant pure human Ep and a polyclonal anti-Ep antiserum as previously described. The standard curve uses an International Ep Standard B, which is provided by the World Health Organization.

RNA isolation and Northern blot analysis. The tumor was frozen at −70°C and total RNA was obtained after direct lysis in 4 mol/L guanidine isothiocyanate followed by extraction with phenol-chloroform. Total RNA (20 μg) was separated by electrophoresis in 1% agarose-formaldehyde gels and transferred to nylon membranes (Nytran; Schleicher and Schuell) as described. Ethidium bromide staining was used to follow proper loading and transfer of the RNA. Hybridization was performed at 42°C for 20 hours in 50% formamide, 5X SSC, 300 μg/mL Na PO₃, pH 6.5, 0.2% sodium dodecyl sulfate (SDS), 2% Denhardt's solution, 5% dextran sulfate, and 200 μg/mL of denatured salmon sperm DNA using 1 × 10⁶ cpm/mL. The probe consisted of a 1.2-kb human Ep cDNA fragment (Genes Institute, Cambridge, MA) labeled with ³²P to high specific activity by the random primer method (Amersham, Illinois). After hybridization the filters were washed with decreasing concentrations of salt to a final wash of 0.1X SSC, 0.1% SDS at 65°C.

RESULTS

Ep levels. The cyst fluid contained markedly elevated Ep levels at 3,650 μU/mL, which is more than 100 times the normal serum concentration. Preoperative and postoperative...
tive serum levels of Ep were 20 and 24 mU/mL, respectively. These values are within our normal range of 5 to 25 mU/mL.

*EP mRNA determination.* Northern blot analysis showed a positive hybridization signal with a human Ep cDNA probe (lane 2, Fig 1) and this confirms the presence of Ep mRNA in the tumor. RNA obtained from a tumor of a patient with cerebellar hemangioblastoma and without erythrocytosis failed to show a positive signal (lane 3 in Fig 1). Equivalent loading of all RNA was confirmed by ethidium bromide staining. The signal of the tumor appears to run slightly below the signal obtained from the RNA of the Ep-producing human hepatoma cell line Hep 3B.

**DISCUSSION**

This patient presented with a typical clinical and radiologic picture of a cerebellar hemangioblastoma in association with mild erythrocytosis. True erythrocytosis was confirmed by the increase of both the RBC mass and total blood volume with a normal plasma volume. Pulmonary hypoxia was excluded by the normal oxygen saturation. The erythrocytosis resolved postoperatively.

The occurrence of erythrocytosis in patients with cerebellar hemangioblastoma is well established. There is a striking male predominance (8:1) that may be explained by a variation in erythropoietin-stimulating activity, hormonal differences, lack of iron stores in females, or other factors. There are no specific histologic features that differentiate between the erythrocytotic and nonerythrocytotic group. The erythrocytosis always resolves with excision of the tumor.

Waldmann et al were the first to demonstrate that the erythrocytosis was a result of erythropoietic stimulation by the tumor. In polycythemic mice, injection of tumor cyst fluid enhanced radiolabeled iron incorporation into erythroid cells. Subsequent investigators confirmed the stimulating activity of the cyst fluid and showed activity in tumor extracts, cerebrospinal fluid, and serum. Using labeled rabbit antiserum to human urinary Ep, hemangioblastomas were shown to contain cells that stain positively for Ep. These investigations suggested that the tumor was a site of synthesis and secretion of Ep.

In this case the demonstration of high Ep levels in the tumor cyst fluid in conjunction with the presence of Ep mRNA in the tumor extract confirms the tumor as the site of Ep production. The size of the tumor Ep mRNA may be slightly smaller than the control Ep mRNA obtained from the hypoxic Hep 3B cells. If so, this could be attributed to differences in the purity, loading, the transcriptional start site, or variation in the length of the poly A tail. The lack of sufficient quantities of RNA precluded further resolution of these questions.

Unfortunately we could not confirm elevation of the serum Ep level. Previous investigators have also had trouble demonstrating elevated Ep activity in the serum of the erythrocytotic patients. This may be a result of intermittent production and/or release of Ep, circadian variation in Ep level, a secondary effect of the shunt in relieving the elevated intracranial pressure (and thus altering the serum level before our determination), or other unknown mechanisms. Although we have not characterized the biologic activity of the Ep, the presence of erythrocytosis suggests that it was active. Whether the ectopic Ep possessed an altered biologic activity was not determined. We believe that the ectopic production (and loss of regulatory control) rather than enhanced activity explains the erythrocytosis.

Ep mRNA production in renal cell carcinoma associated with erythrocytosis has recently been described. This finding and ours clearly demonstrate that the physiologic control of Ep gene expression may be lost in certain tumors. How this control is lost remains speculative. Ep gene rearrangement was not detected in two of three patients studied with renal cell carcinoma. These negative results may indicate that the rearrangement breakpoint was not spanned by the four restriction enzymes used, although this would be unlikely.

Constitutive production of Ep has been described in two viral murine erythroleukemia cell lines. In one of these cell lines rearrangement of the Ep gene is present and it is the rearranged gene that is transcriptionally active. In this instance over-production of Ep may due be to the insertion
of a transcriptionally active gene in close proximity to the Ep gene. A transgenic mouse model of polycythemia provides additional insight into another potential mechanism of gene deregulation. A 4-kb human Ep gene insert containing .4 kb 5' and .7 kb 3' of the Ep gene was expressed in all tissues examined. This insert appears to contain the positive regulatory elements that facilitate expression, but appears to lack the repressor that restricts expression to the liver and kidney. These examples indicate how an alteration in cis, which affects the regulatory sequences of the Ep gene, could result in enhanced Ep expression.

The lack of Ep gene rearrangement in renal cell carci-

nomas would argue against such an alteration in cis. Rather, this would support an abnormality in trans to the gene that is responsible for the inappropriate Ep expression in tumors such as cerebellar hemangioblastoma.

In conclusion, we have demonstrated that the secondary erythropoietin expression of cerebellar hemangioblastoma is a result of tumor synthesis of Ep. The mechanism for altered Ep gene expression in this and other tumors remains speculative.

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