Angiofibroma: A Flow Cytometric Evaluation of 31 Cases

Abstract—Angiofibromas are histologically benign but locally aggressive tumors that often extend beyond the nasopharynx to involve contiguous structures. In addition, attempts to remove the tumors surgically are often frustrated by local recurrences. We studied 31 angiofibromas by flow cytometry to determine if tumor ploidy could be used to identify those tumors that might behave more aggressively. The patients, all males between 5 and 23 years of age, were divided into two groups. The 21 patients with tumors confined to the nasopharynx or paranasal sinuses were regarded as having *local* disease, the ten with tumors involving the infratemporal fossa, orbit, cheek, or intracranial structures were regarded as having *extensive* disease. Of the ten with extensive disease, six had intracranial involvement. All patients were treated with surgery and only one received postoperative irradiation. Follow-up was available in 24, eight of which developed local recurrences (33%). All tumors, including recurrences and those with intracranial extension, were found to be diploid. We therefore conclude that tumor ploidy cannot be used to predict the clinical course of angiofibromas. (*Skull Base Surgery*, Volume 2, Number 4, 1992, p. 195)

Angiofibromas are uncommon, highly vascular, histologically benign but locally aggressive tumors that occur almost exclusively in males, usually between 10 and 20 years of age. Although they arise from the posterolateral nasal wall in the region of the sphenopalatine foramen, they present secondarily in the nasopharynx and are manifested clinically as nasal obstruction and epistaxis.^{1,2} From the nasopharynx, the tumors may spread to involve one or more of the paranasal sinuses, pterygomaxillary and infratemporal fossae, orbit, or cheek. They may even extend intracranially in 10 to 20% of cases.²⁻⁸ Attempts to eradicate the tumors surgically are often unsuccessful, with local recurrences developing in 5 to 25% of all cases.^{2,3,5-8}

Why a tumor that is histologically benign should be so destructive is unknown. To address this issue, we studied a series of 31 angiofibromas by flow cytometry to determine if they exhibited any abnormalities in DNA content and, if so, whether tumor ploidy could be used to target those tumors that might behave more aggressively.

MATERIALS AND METHODS

The Anatomic Pathology files of Presbyterian-University Hospital and the Eye and Ear Institute Pavilion were reviewed for all angiofibromas accessioned from 1960 to 1990. Thirty-one cases were identified. The existing hematoxylin-eosin slides were reviewed by one of us (L.B.) to confirm their authenticity and to select the most representative block for flow cytometric examination.

The medical records were then reviewed for signs and symptoms and extent of disease. Based on initial clinical presentation, patients were divided into two groups. Those with tumors confined to the nasopharynx or paranasal sinus were regarded as having *local* disease, and those with more advanced tumors involving the infratemporal fossa, orbit, or intracranial structures were regarded as having *extensive* disease. Follow-up was obtained from medical records, the attending physician, or, in some instances, by contacting the patient or a family member directly.

For flow cytometry, paraffin-embedded sections of each primary and all recurrent tumors were cut into 50 μm sections and placed in a 10 ml glass centrifuge tube. A 5 μm section was also cut, stained with hematoxylin and eosin and reviewed to verify that tumor cells were present in the sample block. Deparaffinization was done according to the Hedley technique⁹ using two 10-minute incubations in xylene followed by rehydration with decreasing concentrations of ethyl alcohol (100%, 95%, 70%, and 50%). Tissue sections were washed twice in distilled water and incubated at 37°C for 30 minutes with 0.5% pepsin (Sigma Chemical Co., St. Louis, MO), in 0.9% sodium chloride. After continuous agitation in the 37°C water bath, tissue aggregates were dissociated using a manual technique of repeated aspiration with a 21 gauge needle attached to a tuberculin syringe. The specimens were filtered through a 41 μm nylon mesh (Spectrum Medical Industries, Los Angeles, CA). Cell counts were determined (Neubauer chamber), and a Wright's stained cyto-centrifuge slide preparation (Shandon-Lipshaw, Pittsburgh, PA) was prepared and reviewed to ensure a cell yield and single cell suspension.

The DNA procedure was performed on the specimens utilizing a modified Vindelov technique.¹⁰ The staining procedure was one using a combination of propidium iodide (Sigma) (5 $\mu\text{g}/\text{ml}$), RNase (Sigma) (7 U/ml), and NP40 (Calbiochem, San Diego, CA; 1 $\mu\text{l}/\text{ml}$ in Tris/HCL buffer, 0.01 mol/liter pH 8.0). One milliliter of this reagent was added to a tube containing 1 million test cells in suspension, as already described. Previously, cells were incubated for 45 minutes on ice in the dark. Immediately before acquisition of the data, specimens were again filtered through a 5 ml syringe equipped with 41 μm nylon mesh.

A Becton Dickinson FACScan (Mountainview, CA) equipped with a doublet discriminator module and Cell Fit software was used for data acquisition and analysis. At least 20,000 events were collected on each sample using a low flow rate. For analysis purposes, if only one large peak was found, as was the case for all of these samples, it was assumed to be a "normal" diploid G_0/G_1 population.

RESULTS

Clinical

The patients were all males and, at the time of diagnosis, ranged from 5 to 23 years of age (average, 16 years; median, 16.5 years). Twenty-one (68%) individuals were classified as having *local* and ten (32%) had *extensive* disease. Of the ten with extensive disease, six had intracranial involvement.

All presented with episodic epistaxis and nasal obstruction. Those with extensive disease also complained of facial swelling, proptosis, or diplopia.

Treatment and Follow-up

All patients were treated initially with surgery and through a variety of operative approaches that were selected for maximum exposure and complete excision. Only one patient with intracranial extension of tumor that could not be completely removed received postoperative irradiation.

Follow-up, ranging from 8 months to 30 years (average, 10.6 years; median, 6 years) was available in 24 patients. Of these 24, 14 had *local* and ten had *extensive* disease. Five of the 14 patients with local and three of the ten with extensive disease developed recurrences, for an overall incidence of 33% (8 of 24). Of these eight patients, six had one, one had two, and one had three recurrences. All recurrences were manifested anywhere from 2 months to 5 years after treatment.

Pathology

The tumors were all essentially uniform in their microscopic appearance. Each was composed of a cellular fibrous stroma with numerous blood vessels that ranged from sinusoidal to large gaping vascular spaces (Fig. 1). Almost all of the blood vessels were devoid of smooth muscle.

Flow Cytometry

All of the tumors, including all recurrences, were diploid (Fig. 2).

DISCUSSION

Although angiofibromas are benign tumors, they can be locally destructive if not recognized early and treated aggressively. In our study, spanning a course of 30 years, 6 of 31 tumors (19%) had intracranial extension at the time of initial presentation and 8 of 24 patients (33%) available for follow-up experienced local recurrences despite surgical approaches that were selected for total removal. With increased awareness of this uncommon tumor coupled with the routine use of angiography, improved imaging techniques, and advances in skull base surgery, these figures are expected to decline.

During the last decade, flow cytometry has been used to detect abnormalities in DNA content in a variety of human solid tumors. In many of these, particularly cancers of the breast, lung, colon, kidney, bladder, prostate, and ovary, knowing whether the tumor is diploid or aneuploid has proven to be a valuable clinical prognostic indicator.¹¹⁻¹³ However, in other tumors, such as vagal and carotid body paragangliomas, tumor ploidy has no predictive value.^{14,15}

Figure 1. Typical histologic section of an angiofibroma. Note the cellular fibrous stroma and prominent blood vessels devoid of smooth muscle. (Hematoxylin & eosin; $\times 100$.)

Figure 2. DNA ploidy analysis of representative tumor sample. The histogram reveals only a single peak, which is considered to be diploid.

Based on our study of 31 tumors, we conclude that tumor ploidy as determined by flow cytometry cannot be used to predict clinical behavior in patients with angiofibromas. All of our tumors, including recurrences and those with intracranial extension, were diploid.

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