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Increased Expression of Urinary Matrix Metalloproteinases Parallels the Extent and Activity of Vascular Anomalies

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ABSTRACT. *Objective.* **Matrix metalloproteinases (MMPs) and the angiogenic proteins basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) have been implicated in mechanisms of human cancer and metastasis. Assays were conducted on the urine of patients with vascular anomalies (tumors and malformations), relatively common and occasionally life-threatening disorders for which few therapies exist. We sought to determine whether these angiogenesis modulators are present in the urine and whether their expression is associated with the extent and clinical course of the vascular lesion.**

Methods. **A total of 217 patients with vascular anomalies and 74 age-matched control subjects participated. Urinary MMP expression was determined by substrate gel electrophoresis. Urinary bFGF and VEGF levels were measured by enzyme-linked immunosorbent assay. Each patient was assigned to 1 of 2 categories (tumor or malformation) and 1 of 9 specific groups. Extent of the vascular lesion and activity were scored by a blinded clinician.**

Results. **Urinary high molecular weight (hMW) MMPs and bFGF were significantly increased in patients with vascular tumors (53%) and vascular malformations (41%), compared with control subjects (22%). These percentages increased as a function of extent of the lesion and disease activity. hMW MMPs were increased in 4 groups: infantile hemangioma, other vascular neoplasms, lymphatic malformation and capillary-lymphaticovenous malformations, and extensive and unremitting capillary malformation and arteriovenous malformation. No significant differences among the groups were detected for low molecular weight MMPs or VEGF.**

Conclusions. **Expression patterns of hMW MMPs and bFGF in the urine of patients with tumors and malformations are consistent with their different clinical behavior. These data represent the first evidence that MMPs are elevated in the urine of children with vascular anomalies. These data also suggest that the increased expression of urinary MMPs parallels the extent and activity of vascular anomalies in children. In addition to tumors, vascular malformations are angiogenesis dependent, suggesting that progression of a vascular malformation might be suppressed by angiogenic inhibitors, which would target bFGF and MMPs. *Pediatrics* 2005;116:38–45; *vascular anomalies, matrix metalloproteinases, urinalysis.***

ABBREVIATIONS. CM, capillary malformation; LM, lymphatic malformation; VM, venous malformation; AVM, arteriovenous malformation; CLVM, capillary-lymphaticovenous malformation; ECM, extracellular matrix; MMP, matrix metalloproteinase; hMW, high molecular weight; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; CH, congenital hemangioma; IH, infantile hemangioma; ON, other endothelial neoplasms; COMBM, combined malformations; lMW, low molecular weight; OR, odds ratio; CI, confidence interval; PCH, pulmonary capillary hemangiomatosis; NGAL, neutrophil gelatinase–associated lipocalin.

Vascular anomalies comprise 2 main categories: tumors and malformations.[1–3] The biological classification system that distinguishes these, proposed in 1982, is based on physical findings, clinical evolution, and cellular features.[2–4] Characteristic differences have been extended to include radiologic criteria[5,6] and immunohistochemical markers.[7,8]

Vascular tumors are endothelial neoplasms, and infantile hemangioma is the most common type. Vascular malformations are believed to result from the aberrant development of vascular elements during embryogenesis and fetal maturation. These may be single-vessel forms (capillary, arterial, lymphatic, or venous) or combined. The lesions are designated according to the predominant channel type as capillary malformation (CM), lymphatic malformation (LM), venous malformation (VM), arteriovenous malformation (AVM) and complex forms, such as capillary-lymphaticovenous malformation (CLVM; also referred to as Klippel-Trenaunay syndrome).

Despite apparent endothelial quiescence, some vascular malformations can expand rapidly during adolescence or pregnancy, after a surgical procedure, or in response to trauma. Although vascular malformations typically grow at a rate that is commensurate with that of an affected child, we have observed a number of patients in whom there is relentless progression (Fig 1), often resulting in death. No surgical procedures or identified pharmacologic therapies are currently available to treat these individuals. The cellular mechanisms underlying this rapid expansion are unknown. Although expansion of tumors has been documented to be angiogenesis de-

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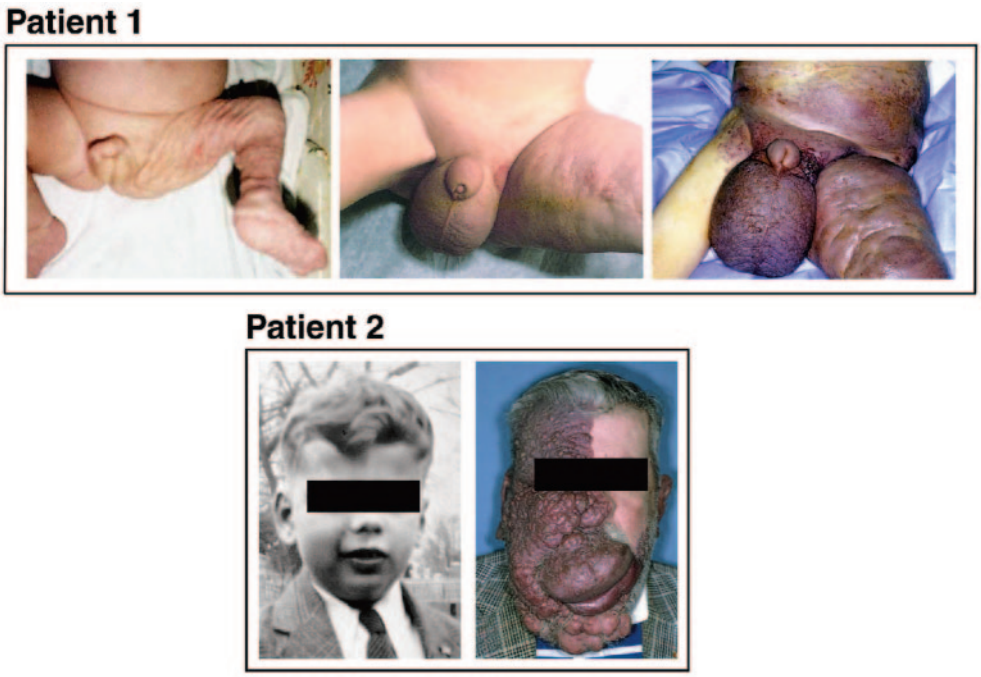

Fig 1. Clinical progression of vascular malformations. Patient 1, a boy with a CLVM, is an example of rapid progression. At 4 weeks of life, his left limb is moderately enlarged compared with the right (left). By 6 years of age, there is increased scrotal and abdominal wall involvement. Amputation of the limb was necessary 6 months earlier because of massive enlargement (center). At 12 years of age, shortly before his death secondary to complications, the malformation is more extensive (right). Patient 2, an example of slow progression of a vascular malformation, was noted at age 5 to have a right facial capillary malformation in association with Sturge-Weber syndrome. He is seen at age 65 with marked fibrovascular thickening and gross distortion of facial structures.

pendent,[9–11] it has not yet been established whether angiogenesis mediates the progression of vascular malformations.

The progression of angiogenesis-dependent disorders depends on the remodeling of extracellular matrices (ECMs) that delimit tissue spaces and surround blood vessels and on the expression of cytokines in promoting neovascularization. Matrix metalloproteinases (MMPs) comprise a multigene family of ~30 metal-dependent enzymes, which collectively degrade components of the ECM to permit formation of new blood vessels. They have been implicated in the pathogenesis of cancer and metastasis, in both experimental models and human neoplasia. Elevated levels of MMPs have been found in the serum and plasma of animals bearing experimental tumors. In human patients, increased serum levels of certain MMPs correlate with the malignant or metastatic phenotype.[12–18]

We previously demonstrated that there is an increased incidence of MMPs in the urine of cancer patients, that biologically active MMP-2 (72-kd gelatinase) or MMP-9 (92-kd gelatinase) is an independent predictor of organ-confined cancer, and that high molecular weight (hMW) species are independent predictors of cancer that has metastasized.[19] In addition, we have shown that urinary basic fibroblast growth factor (bFGF) levels are elevated in patients with a wide spectrum of cancers.[20,21]

bFGF and vascular endothelial growth factor (VEGF) are synergistic angiogenic peptides that stimulate endothelial cell migration, proliferation, and formation of new blood vessels. Increased serum levels have been documented in patients with a wide variety of cancers.[12,15] They have been reported to be predictors of tumor stage and patient survival in certain malignancies.[22]

The purposes of this study were to determine whether urinary MMPs, bFGF, and VEGF are detectable in patients with vascular tumors and malformations and to evaluate whether expression is increased as a function of disease extent and progression. Our findings support consideration of a strategy that uses antiangiogenic therapy to suppress the clinical progression of these diseases.

METHODS

Patient Eligibility and Enrollment

Patients were eligible to participate in this study when they had (1) a readily diagnosed vascular anomaly, (2) no history of therapeutic intervention or other illness for the previous 3 months (including pharmacotherapy, interventional radiologic procedures, or operation), and (3) patient or parental informed consent to participate under the protocol approved by the Institutional Review Board. Control urine samples were provided by healthy siblings of patients in the Vascular Anomalies Center, by children of Children's Hospital employees, and by children at a local child care center, after parental or patient informed consent.

Determination of Extent and Activity of the Vascular Anomaly

Each patient was assigned a diagnosis, on the basis of physical examination and review of clinical and radiographic studies, by a team of Vascular Anomaly Center physicians who were blinded to the results of urine testing. Each patient was assigned to 1 of 2 categories (tumor or malformation) and to 1 of 9 groups within these categories. Groups in the tumor category included congenital hemangioma (CH), infantile hemangioma (IH), and other endothelial neoplasms (ON). In the malformation category, the groups were CM, LM, VM, AVM, CLVM, and combined malformations (COMBM).

Determination of extent and activity for each vascular anomaly was made by a single clinician (S.J.F.) who is familiar with these disorders. Extent was considered (1) limited when it involved <2% of the body surface area or body volume, (2) moderate when it involved 2% to 30% of the body surface area or body volume, or (3) extensive when it involved >30% of the body surface area (Fig 2). Activity was assessed on the basis of the clinical behavior of the lesion during the preceding 6 months as (1) stable when the region grew commensurately with the patient, there was no tissue destruction, and the patient was asymptomatic; (2) active when there was expansion of the anomaly that exceeded growth of the patient, there was tissue destruction, and the patient was symptomatic; and (3) unremitting when there was relentless growth of the anomaly despite all previous medical, surgical, or interventional radiologic therapies.

Urine Collection

Urine samples were processed according to the institutional bioethical guidelines pertaining to discarded clinical material. Freshly voided specimens were divided into aliquots for (1) immediate testing for presence of blood cells, protein, and urinary nitrite, using Ames Multistix 9 reagent strips (Miles, Elkhart, IN); (2) determination of creatinine concentration by the hospital laboratory; (3) enzyme-linked immunosorbent assay testing to quantify levels of urinary bFGF and VEGF (commercially available kits manufactured by R&D Systems, Minneapolis, MN); (4) substrate gel electrophoresis to determine urinary MMP expression profiles; and (5) verification of MMP identity by Western blot analysis.

Substrate Gel Electrophoresis

Urine aliquots for MMP determination were thawed immediately before analysis. Urine zymograms were conducted, as described previously.[23] Both proenzyme and activated proteinases appeared as zones of substrate clearing. Different MMPs were distinguished from each other on the basis of their molecular weights. The identity of known MMPs (MMP-2, 72 kd; and MMP-9, 92 kd) was confirmed by Western blot analysis using anti-MMP antibodies[19] (Oncogene Science, Cambridge, MA). Samples were subjected to incubation in the presence of phenanthroline, an MMP inhibitor, to verify that the proteolytic activities detected were metal-dependent proteinases.

Data Collection and Analysis

Zymograms were processed and evaluated independently by a team of 3 investigators (S.K., Ji.F., and M.A.M.), who were blinded to the clinical status of the patients. Each zymogram was assessed for the presence or absence of the following molecular weight MMPs: >150, 150, 125, 100, 92 (MMP-9), and 72 kd (MMP-2). Of the isoforms that were >92 kd, only the 125-kd isoform has been identified to date.[24] Each sample was scored in a binary manner, ie, presence (positive test) or absence (negative test) for each urinary molecular weight MMP. In addition, it noted whether any hMW form (>92 kd) was present for each patient to facilitate analysis (having 1 broad category of hMW MMPs rather than several different ones).

Quantitative bFGF and VEGF levels were also determined for each sample. Given that there can be wide variation in such levels and that their distributions are non-Gaussian, patients were also scored in regard to whether these test results were abnormal. The urine bFGF was determined to be abnormally elevated (positive test) when it was >4000 pg/mL. The urine VEGF was considered to be abnormally increased (positive test) for values >300 pg/L.

Statistical Analysis

Data were stratified by category (tumor, malformation, or control), clinical group (CH, IH, ON, AVM, VM, LM, MLA, CM, CLVM, or COMBM) within the 2 major categories, extent of the lesion (limited, moderate, or extensive), and activity (stable, active, or unremitting). Data assessed for each category, group, and subgroup (activity or extent) included the percentage of patients who had low molecular weight (lMW) MMPs, hMW MMPs, abnormal bFGF levels (>4000 pg/mL), and abnormal VEGF levels (>300 pg/L). Quantitative bFGF and VEGF levels, as well as age, were also determined and summarized in terms of the median and interquartile range. Each category or group was examined further in terms of percentage of individuals who expressed different types of urinary MMPs (72, 92, 100, 125, 150, or >150 kd).

Baseline characteristics between categories and groups, such as gender, extent, and activity, were evaluated using the Pearson χ^2 test. Data for age and quantitative bFGF/VEGF levels were compared using the Kruskal-Wallis and Mann-Whitney U tests because these variables were not normally distributed.[25] Fisher's exact test was used to compare proportions for MMP expression and cutoff values for bFGF and VEGF. Multiple stepwise logistic regression was used to identify variables that differentiated tumors and malformations from controls with the likelihood ratio test used to assess significance.[26] The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for significant predictors using the normal approximation. For correcting for multiple comparisons, 2-tailed values of $P < .01$ were considered significant. Statistical analyses were performed with the SPSS (version 12.0; SPSS Inc, Chicago, IL) and SAS (version 6.12; SAS Institute, Cary, NC) statistical packages.

Individual groups that did not demonstrate statistically significant increases in MMP, bFGF, or VEGF were stratified by activity and by extent to determine whether more extensive or more active conditions were associated with an increased expression of these markers.

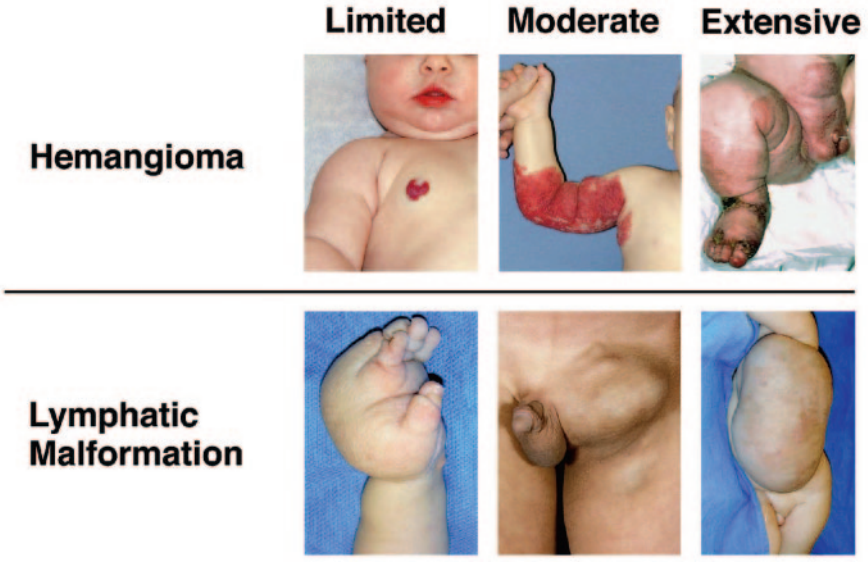

Fig 2. Examples of scoring disease extent for hemangiomas and LMs.

RESULTS

Baseline Characteristics

A total of 291 individuals participated: 217 patients (57 patients with a vascular tumor and 160 patients with a malformation) and 74 control subjects. The tumor category included CH ($n = 8$), IH ($n = 37$), and ON ($n = 12$; single pyogenic granuloma, kaposiform hemangioendothelioma, kaposiform lymphangioendothelioma, pulmonary capillary hemangiomatosis [PCH], angiofibroma, angiomyoma, angiomyolipoma, and epithelioid hemangioma [$n = 5$]). The vascular malformation category included CM ($n = 14$), LM ($n = 47$), VM ($n = 46$), AVM ($n = 25$), CLVM ($n = 20$), and COMBM ($n = 8$). Six patients died as a result of complications secondary to their disease: 5 in the vascular malformation category (12-year-old boy with intraabdominal CLVM, 18- and 22-year-old women with intraabdominal AVMs, and 5- and 14-year-old boys with intrathoracic LM), and 1 in the tumor category (a 12-year-old boy with PCH). All of these patients had extensive, unremitting lesions. To prevent possible confounding of systemic conditions in these critically ill patients on the measurements of hMW MMP, bFGF, and VEGF, we analyzed these patients separately and found that they did not have a disproportionate effect on the overall results.

The vascular tumor and vascular malformation categories were similar with regard to baseline characteristics, although a higher proportion of patients with a malformation had moderate or extensive disease (73% and 11%, respectively), as compared with patients with tumors (60% and 7%, respectively; $P = .01$, Pearson χ^2 test; Table 1). Group baseline characteristics, as shown in Table 2, were also generally similar with the following anticipated exceptions. Compared with control subjects, 47% of whom were girls, there was a higher female preponderance of patients with VM (72%; $P < .01$) and with IH (65%), although the latter did not reach significance ($P = .11$).

Analyses by Major Categories of Vascular Anomaly

Urinary MMP profiles were determined by assaying all urine samples by substrate gel electrophoresis. Representative zymograms from patients with tumors and malformations as well as from control subjects are presented in Fig 3. Fisher's exact test revealed that hMW MMPs were significantly increased in the urine of patients with vascular tumors (53%; $P < .001$) and vascular malformations (41%; $P = .005$), compared with control subjects (22%; Table 3). Abnormal bFGF levels (>4000 pg/mL) were also significantly higher in patients with a vascular tumor (23%; $P = .01$) or vascular malformation (27%; $P = .002$) than in control subjects (8%). These percentages reflect the entire population included, regardless of extent or the severity of the vascular lesion. However, no significant differences were found among proportions of patients with lMW MMPs (MMP-2 or MMP-9) in the tumor or malformation categories. Similarly, the percentages of patients with abnormal urinary VEGF were not elevated (11% of patients for both tumors and malformations; $P = .99$ and $P = .63$, respectively, compared with 10% for control subjects).

Multiple stepwise logistic regression analysis was used to determine the optimal set variables to differentiate both vascular tumors and malformations from control subjects. hMW MMPs were found to be a multivariate predictor of tumor ($P < .001$) and malformation ($P = .002$), whereas bFGF level >4000 pg/L was a multivariate predictor of malformation ($P = .003$) but not of tumor ($P = .15$). Patients with a vascular tumor were estimated to be 4 times more likely to demonstrate a positive hMW MMP, compared with control subjects (OR: 4.0; 95% CI: 2.1–7.6). Patients with a vascular malformation were estimated to be nearly 3 times more likely to have hMW MMPs (OR: 2.8; 95% CI: 1.8–4.7), and 3.5 times more likely to have an abnormal bFGF level (OR: 3.5; 95% CI: 1.6–7.7). VEGF levels >300 pg/L, MMP-2, and MMP-9 were not significant predictors of a tumor or a vascular malformation (all $P > .20$).

Analyses by Groups of Vascular Anomalies

Expression of hMW MMP, bFGF, and VEGF are shown by group in Table 4. Before any stratification for extent or activity, statistically significant increases of hMW MMPs but not of lMW MMPs, bFGF, or VEGF were found in IH (60% of patients; $P < .001$) and other vascular neoplasm (67%; $P = .003$) groups. Percentages of patients with both hMW MMPs and abnormal bFGF tests (but not VEGF) were seen in the lymphatic malformation group (hMW MMP 47% of

TABLE 1. Baseline Patient Characteristics by Disease Category

Characteristic	Controls ($n = 74$)	Tumors ($n = 57$)	Malformations ($n = 160$)
Age, y, median (IQR)*	6.5 (3.5–10.3)	4.4 (2.1–14.4)	9.1 (3.8–14.9)
Gender, female/male†	35/39	34/23	87/75
Disease extent, *n* (%)†			
Limited		19 (33)	35 (16)
Moderate		34 (60)	108 (73)
Extensive		4 (7)	17 (11)
Disease activity, *n* (%)†			
Stable		24 (42)	52 (32)
Active		29 (51)	97 (61)
Unremitting		4 (7)	11 (7)

IQR indicates interquartile range.
* Kruskal-Wallis test, $P = .15$.
† Pearson χ^2 tests: $P = .37$ for gender; $P = .01$ for disease extent; and $P = .41$ for disease activity.

TABLE 2. Baseline Characteristics of Patients by Disease Groups

Characteristic	Tumors (n = 57)			Malformations (n = 160)					
	CH (n = 8)	IH (n = 37)	ON (n = 12)	CM (n = 14)	LM (n = 47)	VM (n = 46)	AVM (n = 25)	CLVM (n = 20)	MIXM (n = 8)
Age, y									
Median	2.8	1.0	15.2	6.4	6.0	10.2	16.5	9.5	8.5
IQR	0.2–10.2	0.3–2.3	12.8–18.1	1.1–19.4	2.6–13.2	5.0–14.8	7.6–23.0	4.6–12.4	4.6–14.1
Gender, female/male	3/5	24/13	7/5	9/5	21/26	33/13	10/15	10/10	2/6
Disease extent, n (%)									
Limited	4 (50)	13 (35)	2 (17)	2 (14)	3 (6)	15 (33)	5 (20)	0 (0)	1 (13)
Moderate	4 (50)	21 (57)	9 (75)	11 (79)	39 (83)	30 (65)	17 (68)	13 (65)	7 (87)
Extensive	0 (0)	3 (8)	1 (8)	1 (7)	5 (11)	1 (2)	3 (12)	7 (35)	0 (0)
Disease activity, n (%)									
Stable	6 (75)	18 (49)	0 (0)	7 (50)	12 (25)	17 (37)	6 (24)	4 (20)	6 (75)
Active	2 (25)	17 (46)	10 (65)	6 (43)	30 (64)	29 (63)	16 (64)	14 (70)	2 (25)
Unremitting	0 (0)	2 (5)	2 (35)	1 (7)	5 (11)	0 (0)	3 (12)	2 (10)	0 (0)

MIXM indicates mixed malformations.

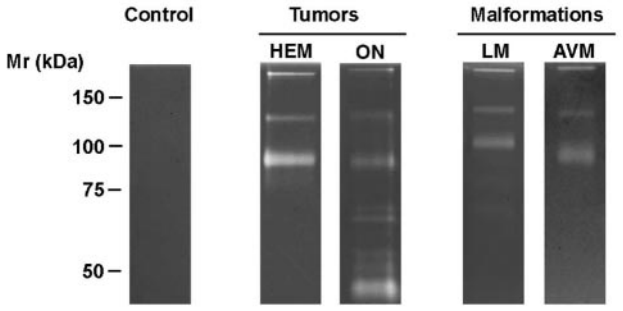

Fig 3. Representative zymograms from individuals with hemangiomas (HEM) display MMP activities at 92 (MMP-9), 125, and >150 kd. In addition to these 3 species, urine samples from individuals with ON also contain lMW species. For the malformation groups, urine samples that were analyzed showed the presence of MMP activities of 100, 125, and >150 kd. Representative urine from a patient with an AVM displayed MMP activities at 92 (MMP-9), 125, and >150 kd.

patients [P = .005]; bFGF >4000 pg/mL 38% [P < .001]) and CLVM groups (hMW MMP 60% of patients [P = .002]; bFGF >4000 pg/mL 50% [P < .001]). In addition, MMP-9 was significantly elevated in the group of other vascular neoplasms (67% of patients; P = .01).

When the remaining groups were stratified by extent and activity of the vascular anomaly, nearly significant increases in hMW MMPs (but not bFGF or lMW MMPs) were documented in patients with moderate or extensive capillary malformation (55% of patients; P = .02, Fisher's exact test) and in patients with extensive and unremitting AVM (100%; P = .01). Urinary MMPs and bFGF were not significantly expressed in patients with VM in any of the extent or activity subgroups.

Stratification by Extent and Activity

The percentages of patients in each category with hMW MMPs and abnormal bFGF and VEGF tests, stratified by disease extent and activity, are shown in Fig 4.

Evaluation of Specific hMW MMP Isoforms Present

Analyses of each MMP-positive sample were performed to determine which specific forms of MMPs (72, 92, 100, 125, 150, or >150 kd) were expressed in each category and group (Fig 3). In both the tumor and the malformation categories, there was increased expression of 100-, 125-, 150-, and >150-kd forms but not of the 72- and 92-kd forms. In fact, the >150-kd form was uniformly expressed whenever increased hMW MMPs were noted.

The 150- and 125-kd bands were present in the urine of patients with IH, ON, and CLVM. Increased expression of the 150-kd form was also noted in the LM group, whereas the 125-kd form was elevated in the AVM group. The 100-kd band was present, with statistical significance, in the urine of patients with IH, CLVM, CM, and LM. The 92-kd species was elevated in the ON and LM groups, whereas the 72-kd species was increased in the CLVM group.

DISCUSSION

We report here, for the first time, that urinary MMPs can be detected in their intact and fully functional forms in patients with both vascular tumors and vascular malformations. This study also demonstrates that urinary bFGF, previously recognized to be increased in tumor patients,[20,21] is also elevated in patients with vascular malformations. Finally, we show that the increased expression of both hMW MMPs and bFGF correlates with both extent and the progression in patients with a vascular anomaly.

It is widely appreciated that the activity of the MMP family of proteolytic proteins is the rate-limiting step in degradation of the ECM. Given the extent of tissue remodeling in tumors, both vascular and stromal, it is not surprising that these enzymes play an important role. However, the presence of these enzymes, together with bFGF, in the urine of children with a vascular malformation is an exciting and unpredicted finding.

Previous studies have demonstrated that urinary MMP-2 and MMP-9 are detected in the urine of healthy children as a consequence of the normal tissue remodeling associated with growth and development.[27] This finding is supported by our current study, in which we found no correlation between urinary MMP-2 or MMP-9 and extent or activity. We previously reported that, in adulthood in contrast to adolescence, MMPs are not routinely detected in the urine of healthy adults, regardless of age or gender.[19]

TABLE 3. Results by Disease Category

Variable	Controls (n = 74)	Tumors (n = 57)	Malformations (n = 160)	P	
				Controls Versus Tumors	Controls Versus Malformations
hMW MMP, n (%)	16 (22)	30 (53)	65 (41)	<.001*	<.01*
72-kd species, n (%)	32 (43)	31 (54)	78 (49)	.22	.48
92-kd species, n (%)	22 (30)	26 (46)	69 (43)	.07	.06
bFGF, pg/mL					
Median (IQR)	999 (999–2096)	1871 (999–3874)	1695 (999–4280)	<.01*	<.01*
>4000, n (%)	6 (8)	13 (23)	43 (27)	.02*	<.01*
VEGF, pg/L					
Median (IQR)	96 (54–171)	107 (63–187)	102 (53–172)	.51	.87
>300, n (%)	7 (10)	6 (11)	17 (11)	.99	.63

Fisher's exact test was used to compare proportions, and the Mann-Whitney U test was used to assess differences in medians.
* Statistically significant.

TABLE 4. Results by Disease Group

Variable	Controls (n = 74)	CH (n = 8)	IH (n = 37)	ON (n = 12)	CM (n = 14)	LM (n = 47)	VM (n = 46)	AVM (n = 25)	CLVM (n = 20)	MIXM (n = 8)
hMW MMP	16 (22)	0 (0)	22 (60)	8 (67)	6 (43)	22 (47)	13 (28)	9 (36)	12 (60)	3 (38)
P	—	.34	<.001*	<.01*	.10	<.01*	.50	.19	<.01*	.40
72-kd species	32 (43)	2 (25)	22 (60)	7 (58)	7 (50)	14 (30)	24 (52)	15 (60)	15 (75)	3 (38)
P	—	.46	.11	.37	.77	.18	.35	.17	.02	.99
92-kd species	22 (30)	1 (13)	17 (46)	8 (67)	7 (50)	14 (51)	18 (39)	9 (36)	9 (45)	2 (25)
P	—	.43	.10	.02*	.21	.02*	.32	.62	.62	.99
bFGF >4000 pg/mL	6 (8)	1 (12)	8 (22)	4 (33)	2 (14)	18 (38)	7 (15)	4 (16)	10 (50)	2 (25)
P	—	.53	.07	.03*	.61	<.001*	.24	.27	<.001*	.17
VEGF >300 pg/L	7 (10)	0 (0)	4 (11)	2 (17)	1 (7)	7 (15)	2 (4)	1 (4)	5 (25)	1 (12)
P	—	.99	.99	.61	.99	.39	.48	.67	.12	.58

Data represent number of patients (%). Fisher's exact test was used to compare proportions between each group and control subjects.
* Statistically significant.

Rather, when MMP-2 and MMP-9 are present in the urine of adults, they are multivariate predictors of organ-confined cancer and metastatic malignancy. Therefore, it is not surprising that the only group to demonstrate a significant increase in lMW MMPs (specifically MMP-9) was the group of other vascular tumors.

The identity of the hMW MMP forms is currently under study. We recently reported that the urinary MMP species of 125 kd is a complex of MMP-9 and neutrophil gelatinase–associated lipocalin (NGAL).[24] We demonstrated that the complexing of NGAL with MMP-9 has the function of protecting MMP-9 from autodegradation, thereby preserving its full degradative activity. It is interesting that we have detected MMP-9/NGAL more frequently in the urine of adults who have metastatic cancer than other types of the disease. We believe that the invasive capacity of MMP-9 is enhanced when it is complexed to NGAL and that this is associated with metastasis. We suggest that these novel hMW urinary MMPs, perhaps including MMP-9/NGAL, are a feature of extensive and unremitting vascular malformations.

The clinical behaviors of the different vascular entities paralleled our findings in the urinary proteins. For example, a congenital hemangioma is fully formed at birth and begins to involute immediately, whereas the common hemangioma develops postnatally and grows rapidly during the first year. The infants with CH (n = 8) showed no increase in either hMW MMPs or bFGF; however, those with IH had increases in both.

The propensity for a vascular malformation to progress varies according to the type of abnormal channel or flow characteristics. AVMs are notorious for their tendency to destroy tissue, whereas VMs typically exhibit a slow, gradual expansion with little or no damage to surrounding tissue. In addition, increased levels of MMP-9 in the tissues of adult patients with brain AVMs were reported recently.[28] These clinicopathologic observations parallel our findings of elevated bFGF and hMW MMPs in AVM patients (with affected patients increasing as a function of extent and clinical stage). In our study, no significant increases of any angiogenic markers were found in patients with VM, although this does not preclude the possibility that other growth factors may play a role in this disease.

We did not identify any patterns of MMP expression that characterized subsets or profiles of specific MMP forms that could reliably distinguish groups or categories. In all instances in which hMW forms were expressed with statistical significance, the >150-kd MMP was present. Expression of the 150-, 125-, and 100-kd enzymes was more variable: increased in some patient groups but not all. It remains possible, however, that "isoform patterns" will emerge as results from more patients are added and analyzed. Whereas most malformations enlarge commensurately with the growth of a child, some expand disproportionately (Fig 1). During the time course of this study, in fact, more patients died from complications of a vascular malformation than as a result of a vascular tumor. Patients with an unremitting vascular malformation were found uniformly to express hMW MMPs (n = 16; P < .001), and 73% had an

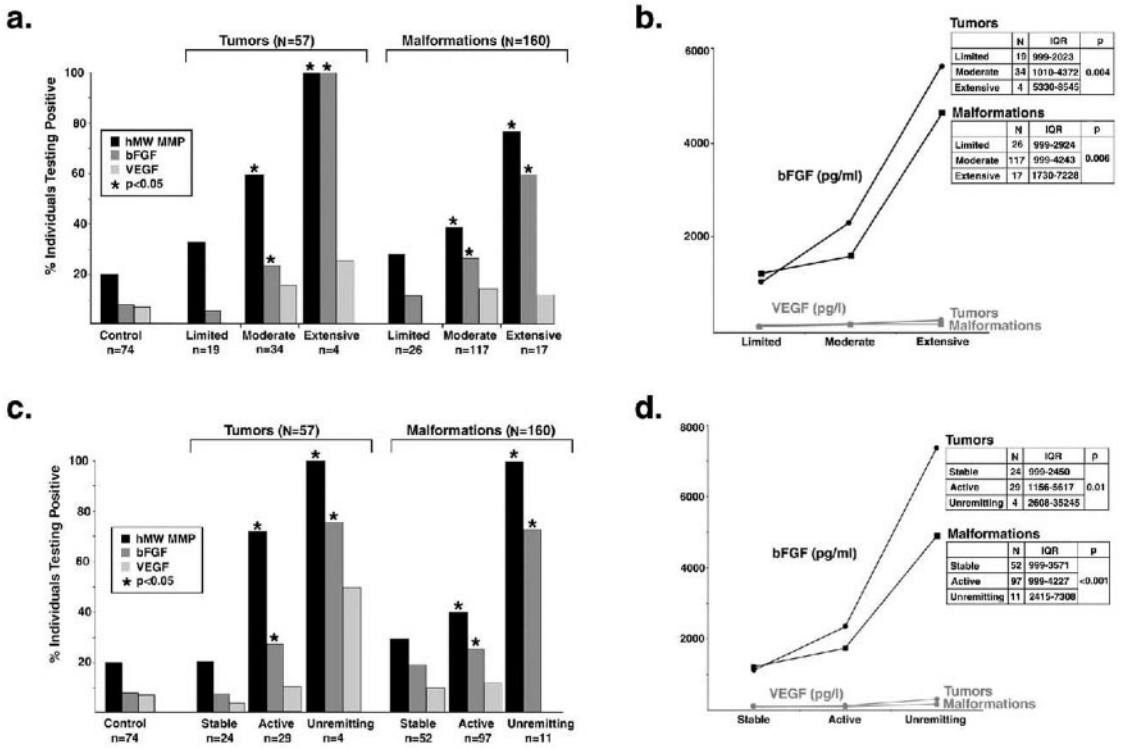

Fig 4. Expression of urinary hMW MMPs, bFGF, and VEGF as a function of disease extent and activity. Significantly higher percentages of patients with moderate and extensive tumors and malformations had urinary hMW MMPs and abnormal bFGF levels (>4000 pg/mL) compared with control (a). For tumors and malformations, a significant increase was observed in bFGF level (pg/mL) but not VEGF level (pg/L), according to disease extent (b). Significantly higher percentages of patients with active and unremitting tumors and malformations had urinary hMW MMPs and abnormal bFGF levels (>4000 pg/mL) compared with control (c). For tumors and malformations, a significant increase was observed in bFGF level (pg/mL) but not VEGF level (pg/L), according to disease activity (d).

increased urinary bFGF ($n = 16$; $P < .001$). There are currently no identified therapies, pharmacologic or surgical, to slow or halt the progression of disease in these individuals. Our work suggests that the use of angiogenesis inhibitors that are designed to target MMPs or bFGF may represent a therapeutic strategy to suppress an expanding vascular malformation.

Although this analysis focused on single urinary samples from individual patients, we do have anecdotal evidence of the elimination of hMW MMP isoforms after therapy in 2 patients (Fig 5). The first child was an infant in whom there was disappearance of hMW MMP bands after accelerated regression of a common hemangioma with oral corticosteroid for 6 months. The second child presented to our clinic as a neonate with an extensive axillary lym-

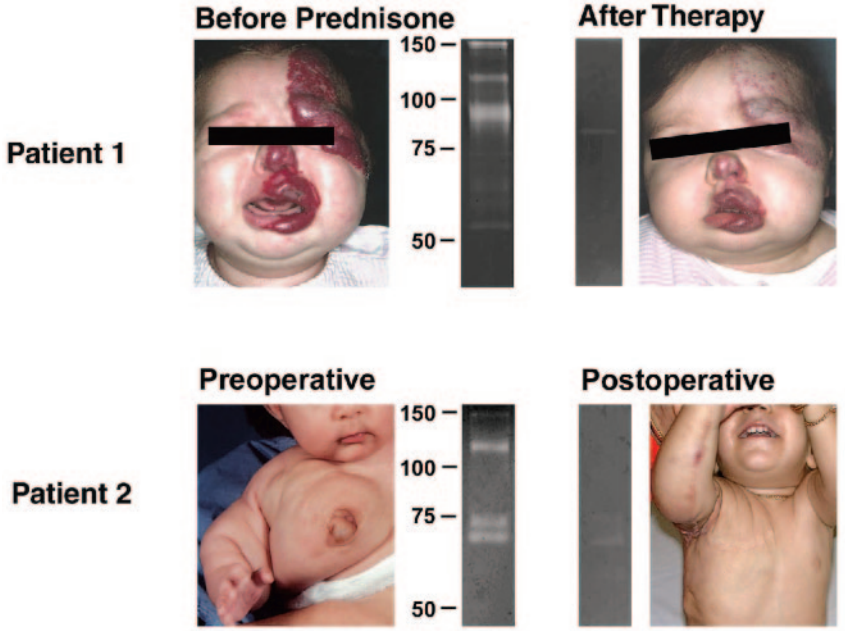

Fig 5. Changes in urine MMP expression profiles in 2 patients after therapy. In the urine of patient 1, there was a significant reduction in hMW isoforms after treatment of her hemangioma with corticosteroids. In the urine of patient 2, a comparable change was seen after extensive surgical resection of an LM that affected the right upper extremity and chest wall.

phatic malformation and, after comprehensive surgical resection, hMW bands disappeared from the urine. We recently reported success in treating a 20-year-old male patient with PCH for 19 months of therapy with doxycycline, an angiogenesis inhibitor. This treatment resulted in the normalization of pulmonary function and restoration of normal urine bFGF levels.[29]

Enlargement of a vascular malformation, such as LM or AVM, in the months after surgical resection could be tested. In addition, proteomics will permit the broad direct detection and identification of other aberrant urinary proteins. Clinical trials are needed to determine whether angiogenesis inhibitors can suppress clinical progression of tumors and vascular malformations.

This study confirms previous reports that bFGF, VEGF, and MMPs can be identified and measured independently in urine.[19–21] It further introduces the concept of urinary "angiogenic profiling," in which multiple angiogenesis-related molecules are assayed in the same sample. The identification of these proteins should provide insights into the pathophysiologic bases for these vascular disorders and may be used together with clinical markers of remission or exacerbation in response to treatment. This approach likely will yield specific profiles of endogenous angiogenesis stimulators and inhibitors that characterize different types of vascular anomalies.

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Increased Expression of Urinary Matrix Metalloproteinases Parallels the Extent and Activity of Vascular Anomalies

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