Extracts from tumors causing oncogenic osteomalacia inhibit phosphate uptake in opossum kidney cells

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Abstract

In oncogenic osteomalacia (OOM), a tumor produces an unknown substance that inhibits phosphate reabsorption in the proximal tubules. This causes urinary phosphate wasting and, as a consequence, hypophosphatemic osteomalacia. To characterize this poorly understood biological tumor activity we generated aqueous extracts from several OOM tumors. Extracts from three of four tumors inhibited, dose- and time-dependently, ³²P-orthophosphate uptake by opossum kidney (OK) cells; maximum inhibition was about 45% of untreated control. Further characterization revealed that the factor is resistant to heat and several proteases, and that it has a low molecular weight. The tumor extracts also stimulated cAMP accumulation in OK cells, but not in osteoblastic ROS 17/2.8 and UMR106 cells, or in LLC-PK1 kidney cells expressing the parathyroid hormone (PTH)/PTH-

related peptide receptor or the PTH-2 receptor. HPLC separation of low molecular weight fractions of the tumor extracts revealed that the flow-through of all three positive tumor extracts inhibited ³²P uptake and stimulated cAMP accumulation in OK cells. Additionally, a second peak with inhibitory activity on phosphate transport, but without cAMP stimulatory activity, was identified in the most potent tumor extract. We have concluded that several low molecular weight molecules with the ability to inhibit phosphate transport in OK cells can be found in extracts from OOM tumors. It remains uncertain, however, whether these are related to the long-sought phosphaturic factor responsible for the phosphate wasting seen in OOM patients.

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Introduction

Parathyroid hormone (PTH) and the biologically active vitamin D metabolite, 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃) are the two major hormonal regulators of phosphate homeostasis. PTH decreases the tubular reabsorption of phosphate by accelerating the degradation of the type II sodium-dependent phosphate transporter (NPT-2) (Murer *et al.* 1999), the most important phosphate transporter in the proximal tubules. 1,25(OH)₂D₃ does not have a direct role in renal phosphate handling but enhances phosphate uptake from the gut. However, changes in the levels of these two hormones do not explain the dramatic increase in renal reabsorption of phosphate in

response to phosphate starvation, suggesting that additional factors are involved in the regulation of this mineral (Drezner 1996).

Studies of X-linked hypophosphatemic rickets (XLH), a rare genetic disorder, and the corresponding animal models, the *gy* and *hyp* mice, have recently yielded new important insights into the regulation of phosphate homeostasis (Econs 1999, Tenenhouse 1999, Drezner 2000). XLH is a renal phosphate wasting disease with associated hypophosphatemic rickets. Characteristic biochemical findings include hyperphosphaturia, hypophosphatemia, and inappropriately low circulating levels of 1,25(OH)₂D₃ (Tenenhouse 1999). The mutated gene responsible for the disease, *PHEX*, encodes a membrane-

bound neutral endopeptidase that is not expressed in the kidney (Ruchon *et al.* 1998, Du *et al.* 1996, Beck *et al.* 1997). To explain how mutations in this gene can cause phosphaturia it was necessary to invoke the actions of a yet-unidentified hormonal factor, provisionally referred to as phosphatonin, with the ability to decrease directly renal tubular phosphate reabsorption in the kidney (Econs & Drezner 1994). The currently prevailing hypothesis thus suggests that *PHEX* inactivates phosphatonin and that loss-of-function mutations in this enzyme increase the levels of phosphatonin in the circulation.

Similarly, increased concentration of a phosphaturic factor has been implicated in the pathogenesis of oncogenic osteomalacia (OOM), an acquired condition characterized by phosphopenic osteomalacia due to renal phosphate wasting (Nelson *et al.* 1997, Drezner 2000). Patients affected by OOM exhibit biochemical characteristics that are similar to those observed in XLH patients. The disease is usually caused by inconspicuous benign tumors that may be found in a variety of different locations. If the tumors are localized and successfully removed, there is prompt improvement of the symptoms, and phosphate homeostasis is rapidly normalized. This strongly suggests that a tumor-derived humoral factor, such as the proposed phosphatonin, causes phosphate depletion by acting on the proximal tubule cells to increase urinary phosphate excretion. Several groups have attempted to characterize the putative phosphaturic factor by different approaches. For example, extracts from OOM tumors were shown to be phosphaturic when injected into dogs, rats or mice, and to inhibit the production of $1,25(\text{OH})_2\text{D}_3$ in primary kidney cell cultures (Aschiner *et al.* 1977, Lau *et al.* 1979, Popovtzer 1981, Miyauchi *et al.* 1988). Others have shown that conditioned medium from primary cultures of tumor cells inhibits phosphate uptake by opossum kidney (OK) cells (Cai *et al.* 1994, Wilkins *et al.* 1995, Nelson *et al.* 1996). However, attempts to elucidate the chemical nature of the OOM factor have yielded conflicting results. For example, the proposed entity has been found to be heat labile or heat resistant (Miyauchi *et al.* 1988, Cai *et al.* 1994, Wilkins *et al.* 1995, Nelson *et al.* 1999), to be trypsin sensitive or trypsin insensitive (Miyauchi *et al.* 1988, Cai *et al.* 1994, Wilkins *et al.* 1995, Nelson *et al.* 1996) and to have a molecular weight of 56 kDa, 8–25 kDa or less than 5 kDa (Cai *et al.* 1994, Rowe *et al.* 1996, Nelson *et al.* 1999).

Thus, based on the similarities of the laboratory and clinical findings in patients affected by XLH and OOM, it appears plausible that both disorders are caused by increased production/activity of the same phosphaturic factor. However, the identity of this proposed factor remains elusive. To gain further insights into its nature, we generated and characterized extracts from several different OOM tumors.

Materials and Methods

Materials

Human [Y34]PTH(1–34)amide (subsequently referred to as PTH) was synthesized by the biopolymer synthesis facility at Massachusetts General Hospital (Boston, MA, USA). Centricon and microcon spin filters were purchased from Millipore (Bedford, MA, USA). Sequencing grade trypsin was purchased from Promega (Madison, WI, USA). Papain and proteinase K were from Roche Molecular Biochemicals (Mannheim, Germany), the C8 Brownlee microbore column (2.1 × 100 mm) was from Perkin-Elmer Applied Biosystems (Foster City, CA, USA). Dulbecco's minimal essential medium (DMEM)/HAM-F12 and fetal calf serum (FCS) were from Life Technologies (Grand Island, NY, USA), ^{32}P -orthophosphate (2 mCi/ml) was from New England Nuclear (Boston, MA, USA), and the BCA protein assay was from Pierce (Rockford, IL, USA). All other reagents were of the highest available purity and were obtained through Fisher Scientific (Pittsburgh, PA, USA).

Patients and tissue extraction

Extracts were generated from four different tumors, some of which have been described previously; a mandibular tumor from a 31-year-old Korean female (Yang *et al.* 1997) (tumor A), a tumor in the adductor channel of the left thigh from a 31-year-old Swedish male (Nelson *et al.* 1998) (tumor B), a tumor which was removed in March 1998 from the sole of the foot of a German female (tumor C), and a tumor from the left thigh of a 54-year-old Japanese male (Miyauchi *et al.* 1988) (tumor D). All patients had presented with biochemical findings characteristic of OOM, which dramatically improved after surgery. All tumors were instantly frozen after removal from the patients and stored at -70°C until used in this study. Pieces of tumor or control tissues (parathyroid, liver, and kidney) (75–250 mg) were removed from frozen samples and were homogenized on ice using a glass tissue grinder in 100–500 μl ice-cold normal saline. The homogenates were transferred into microfuge tubes and spun at 4°C (20 000 g; 5 min). The supernatants were transferred into fresh tubes and protein content was measured using the Pierce BCA protein assay; bovine serum albumin was used as a standard. The protein concentration was subsequently adjusted to 2 mg/ml.

Phosphate uptake assay

OK cells were grown in DMEM/HAM-F12 supplemented with 5% FCS and 1% penicillin/streptomycin (5000 IU/ml, 5 mg/ml). Cells were trypsinized and 5×10^4 cells were then seeded into the inner 24 wells of a 48-well tissue culture plate. After reaching confluency,

the cells were cultured for 2 more days before performing experiments. To assess phosphate uptake, the medium (two wells/data point) was replaced with medium containing tumor extracts (concentration: 2.5%, v/v; unless otherwise indicated) or vehicle (equal volume of saline). After 12 h, cells were rinsed twice with uptake buffer (150 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 5 mM glucose, and 10 mM cyclohexylaminopropane sulfonic acid, pH 9.5), before adding 100 µl/well of the same buffer supplemented with 5×10^5 c.p.m. of ³²P-orthophosphate and 0.2 mM KH₂PO₄. After 5 min at 37 °C, the reaction was interrupted by rapidly aspirating the buffer and replacing it with 250 µl ice-cold 'STOP' solution (5 mM sodium arsenate and 150 mM choline chloride, pH 7.4). The cells were subsequently lysed with 0.5 M NaOH (250 µl) and total radioactivity of the lysate was measured in a liquid scintillation counter. In protease inactivation experiments, the extracts were pretreated using the following experimental conditions: trypsin (50 µg/ml), 37 °C, 12 h in 10 mM Tris, pH 8.0; papain (50 µg/ml), 37 °C, 12 h in 10 mM Tris, pH 7.0; proteinase K (100 µg/ml), 50 °C, 12 h in 10 mM Tris, pH 8.0 with 0.1% SDS.

Stimulation of cAMP accumulation

Confluent OK cells were rinsed with ice-cold phosphate-buffered saline and 100 µl IBMX buffer (DMEM containing 2 mM isobutylmethylxanthine (IBMX), 1 mg/ml bovine serum albumin and 35 mM Hepes-NaOH, pH 7.4) containing either tumor extract (2.5%, v/v), PTH (1 µM) or vehicle (normal saline 2.5%, v/v). The same procedure was used to stimulate cAMP accumulation in ROS 17/2.8, UMR106, or LLC-PK1 cells stably expressing either the PTH/PTH-related protein (PTHrP) or the PTH-2 receptor (Carter *et al.* 1999). After incubating the plates for 1 h at room temperature, the buffer was completely removed, the plates were placed on powdered dry ice, and intracellular cAMP was extracted by adding 250 µl 10 mM HCl. The cell extracts were analyzed in duplicates for cAMP content by radioimmunoassay (RIA) as described (Carter *et al.* 1999).

HPLC fractionation

About 100 µl of each crude tumor extract was diluted with H₂O (1:1) before centrifugation (14 000 g, 45 min at 4 °C) through a microcon filter with a cut-off of 3 kDa. After lyophilization, the flow-through was reconstituted with 100 µl H₂O/0.1% trifluoroacetic acid (TFA) and the entire sample was injected onto a C8 microbore column (2.1 × 100 mm); the following gradient and solvents were used: initial condition, 98% solution A (0% acetonitrile (ACN), 0.06% TFA in H₂O) and 2% solution B (80% ACN, 0.05% TFA in H₂O) followed by a 2–95% solution B gradient over 80 min; the flow rate was 100 µl/min,

200 µl were collected per fraction. After lyophilization, fractions were dissolved in 200 µl culture medium and the reconstituted samples (100 µl/well) were tested for inhibition of phosphate uptake using OK cells as described above.

Results

To determine whether OOM tumors produce a factor(s) that inhibits phosphate uptake in OK cells, we generated several independent extracts from four different tumors and tested their ability to inhibit the uptake of ³²P-orthophosphate by these cells. Three extracts induced significant inhibition of ³²P transport but their individual efficacy varied considerably. For example, extract A was most efficacious and reduced ³²P uptake to $42 \pm 5\%$ of the uptake in untreated control cells (Fig. 1A). This effect was similar to that of extracts derived from a parathyroid adenoma. Extracts from tumors B and C were less efficacious and inhibited ³²P uptake to only $69 \pm 5\%$ and $71 \pm 5\%$ of control respectively. In contrast, extracts from tumor D inhibited only to $86 \pm 4\%$. We also generated extracts from kidney and liver, and found that these control extracts displayed an inhibitory activity that was comparable with that observed with extract D (Fig. 1A).

To explore the possibility that the tumor extracts mediated their activity through a G protein-coupled receptor, we measured cAMP accumulation in response to the different extracts. The three tumor extracts that had shown significant inhibition of ³²P uptake also induced cAMP accumulation in the OK cells whereas extracts from liver, kidney, and tumor D did not (Fig. 1B). As expected, a strong cAMP response was induced by the parathyroid adenoma extract. To determine whether the cAMP response could be observed in cells other than the OK cells, we tested two other clonal osteoblast-like cell lines, ROS 17/2.8 and UMR106, as well as LLC-PK1 cells stably expressing either the recombinant human PTH/PTHrP receptor or the human PTH-2 receptor (Carter *et al.* 1999). When challenged with PTH, each of these cell lines responded with an increase in cAMP accumulation, but not when treated with any of the tumor extracts (data not shown). In addition, UMR106 cells responded to isoproterenol and dopamine, and ROS 17/2.8 cells responded to prostaglandin E2 and isoproterenol.

To further characterize the factor(s) that inhibits ³²P uptake, the most potent extract was studied in more detail in dose-response and time-course experiments. Tumor-induced inhibition of phosphate uptake was already apparent after 2 h and reached maximal levels after 12 h which was very similar to the effects induced by PTH (Fig. 2A). We next compared the inhibition induced by different dilutions of extract A with that induced by increasing concentrations of PTH, and found

Figure 1 Effects of crude aqueous extracts from OOM tumors on phosphate transport in OK cells. OK cells were grown to confluency before treatment with PTH (1 μ M) or tissue extracts (2.5%, v/v). (A) After a 12-h incubation, uptake of 32 P-orthophosphate was measured over a 5-min period as described in Materials and Methods. Inhibition of phosphate uptake is expressed as percent of the uptake of non-treated control (Ctr) cells. *Not significantly different from untreated control using ANOVA. (B) After a 1-h incubation, intracellular cAMP was extracted from the cell layer and measured by RIA. Results are expressed as fold over basal cAMP accumulation in vehicle-treated cells. All data are shown as means $\pm$ S.E.M. and are derived from at least six independent experiments for (A) and from two independent experiments for (B). PTH: [Tyr34]hPTH(1–34)amide (1 μ M); PT: extract from parathyroid adenoma (2.5%); A, B, C, and D: extracts from the different tumors (2.5%); KID: kidney extract (2.5%); LIV: liver extract (2.5%).

that approximately 0.6% (v/v) of this extract was as potent as 10 nM PTH. Furthermore, the dose–response curves for PTH and extract A ran in parallel over the entire range tested (Fig. 2B).

In an attempt to partially purify the activity identified in the tumor extracts, we first used centrifugation filters with different molecular weight cut-offs. Stimulation of cAMP accumulation and inhibition of 32 P uptake was primarily recovered in the flow-through, even when using filters

with a 3 kDa cut-off. These findings suggested that both activities are due to low molecular weight compounds. Fractionation of the flow-through by C8 microbore HPLC revealed two peaks with biological activity when tested in the OK cell assay. For all three tumor extracts, fraction no. 2 (i.e. the flow-through) stimulated the accumulation of cAMP and inhibited 32 P uptake. For extract A, 32 P uptake was also inhibited by material eluting in fraction nos 23–24; these fractions failed to stimulate cAMP accumulation (Fig. 3A and B).

To further characterize the chemical nature of the biologically active compounds detected in extract A, aliquots were either heated to 99 °C or treated with one of several different proteases. Heating for 1 h failed to diminish the activity of extract A (Fig. 4). Also, the activity of synthetic PTH or parathyroid extracts remained unaffected. The tumor extract was furthermore resistant to trypsin, proteinase K, and papain, although these enzymes efficiently abolished the capacity of synthetic PTH and parathyroid extracts to reduce 32 P uptake by OK cells (Fig. 4); despite proteinase or heat treatment, cAMP accumulation by the unfractionated tumor extracts remained unchanged (data not shown).

Discussion

Tumors that cause OOM secrete a factor(s) that acts directly or indirectly on the kidney to reduce, through yet unknown mechanisms, the reabsorption of phosphate in the proximal tubules. This OOM-derived factor is thought to act through a specific cell surface receptor on the kidney cells and activation of this receptor may either accelerate the degradation of NPT-2 protein or its mRNA and/or diminish new synthesis. Based on this hypothesis and knowledge of the biochemical findings in OOM patients, different cell-based *in vitro* assays have been devised to assess the effects of the phosphaturic factor. For example, the inappropriately low serum concentration of 1,25(OH) $_2$ D $_3$ in OOM patients indicates that phosphatonin inhibits the renal 1 α -hydroxylase. Accordingly, OOM tumor extracts were shown to reduce the 1 α -hydroxylation of 25 hydroxyvitamin D $_3$ in primary renal cell cultures (Miyachi *et al.* 1988). Another approach, which was used in the present study, takes advantage of NPT-2-dependent transport of radioactive phosphate across the plasma membrane of OK cells. These cells express, in addition to NPT-2, the PTH/PTHrP receptor. When activated by PTH (or PTHrP), events downstream of this G protein-coupled receptor lead to the rapid degradation of this sodium-phosphate cotransporter (Murer *et al.* 1999). Consequently, OK cells respond to challenge with PTH (or PTHrP) with a reduction in 32 P uptake, thus mimicking the *in vivo* effects of the hormone. Several groups have previously shown that medium conditioned by cells derived from OOM tumors, as well as

Figure 2 Time-course and dose-response effects of the extract A in comparison with PTH. OK cells were grown to confluency before treatment with PTH or extract A. After the indicated time-periods, uptake of ^{32}P -orthophosphate was measured over a 5-min period as described in Materials and Methods. Inhibition of phosphate uptake was expressed as percent of the uptake in non-treated control (Ctr) cells. The graphs show the means $\pm$ S.E.M. of uptake in duplicate wells in a single representative experiment. Similar results were observed in two additional experiments. (A) Time-course experiment: OK cells in 48-well plates were rinsed and fresh medium was added. At the indicated times before measuring phosphate uptake, cells were treated with 1 μM PTH(1–34) ($\square$) or 2.5% of extract A ($\bullet$). (B) Dose-response experiment: [Tyr34]hPTH(1–34)amide ($\square$) and extract A ($\bullet$) were serially diluted and OK cells were treated for 12 h before measuring uptake of ^{32}P -orthophosphate.

serum from either OOM patients or *hyp* mice, inhibit ^{32}P uptake by OK cells, thus supporting the hypothesis that these cells also express the putative phosphatonin receptor (Cai *et al.* 1994, Lajeunesse *et al.* 1996, Nelson *et al.* 1996, 1998).

We had the opportunity to characterize, under standardized conditions, aqueous extracts from four different tumors that clearly gave rise to OOM. This allowed us to extend previous studies which had investigated, in most cases, only a single tumor and had provided conflicting results. For example, some investigators observed an inhibition of phosphate uptake by OK cells (Cai *et al.* 1994, Wilkins *et al.* 1995, Nelson *et al.* 1998), an inhibition of 1 α -hydroxylase activity (Miyauchi *et al.* 1988), or phosphaturia in live animals (Miyauchi *et al.* 1988), while others failed to detect any *in vitro* changes (Shane *et al.* 1997). These inconsistencies may be due to differences in experimental techniques or could be related to heterogeneity among or within different tumors. Thus, it is not surprising that we also found strong variability regarding the inhibition induced by the different tumors. For

example, extract A produced consistent inhibition of ^{32}P uptake that was similar to the effect induced by synthetic PTH. In contrast, extracts from two other tumors, B and C, had only a weak effect and a fourth extract, derived from tumor D, showed no effect at all. These findings could imply that the phosphaturic entity differs among tumors, that different tumors produce varying quantities of the same substance, that the phosphaturic factor is produced only in a small portion of each tumor, or that the biologically active factor requires co-factors. It is also possible that prolonged storage, even at -80°C , can cause a loss of biological activity and this might explain the almost complete lack of inhibitory activity in tumor D.

The dose-response and time-course of the inhibition induced by extract A was similar to the inhibition induced by PTH. This could indicate that the events induced by PTH and the OOM factor converge on a common downstream signaling pathway, supporting the conclusion that the effects of extract A are mediated by a receptor-coupled mechanism. This raises the question as to whether tumor extracts and PTH activate a common second

Figure 3 HPLC fractionation of tumor extracts provides evidence for two distinct factors that inhibit phosphate uptake by OK cells. Size-selected tumor extracts (<3 kDa) were fractionated on a C8 microbore HPLC column and the different fractions were assayed for their ability to inhibit ^{32}P uptake and to stimulate cAMP accumulation. (A) One half of each collected fraction was lyophilized and subsequently dissolved in 200 μl culture medium. OK cells in duplicate wells were then incubated with the reconstituted fractions for 12 h before determining the uptake of ^{32}P -orthophosphate over a 5-min period. (B) The other half of each collected fraction was lyophilized and dissolved in 200 μl culture medium containing IBMX. Duplicate wells with OK cells were then incubated with the reconstituted fractions for 1 h and the intracellular cAMP accumulation was determined by RIA. The means of the duplicate data points are plotted in the graphs and the arrows indicate the position of peaks with significant activity. Solid line (■): tumor A; dashed line: tumor B (▲) and dotted line: tumor C (▼).

messenger pathway. Previously, conflicting results have been reported regarding the ability of tumor derivatives to stimulate cAMP accumulation. Seshhadri *et al.* (1985)

found a potent stimulatory effect of three tumor extracts on the accumulation of cAMP in chicken renal plasma membranes and Nelson *et al.* (1996) found that media

Figure 4 Protease and heat treatment of extract A in comparison with synthetic PTH and parathyroid extract. Extracts from tumor A and a parathyroid adenoma (1:25% of the final volume of medium) were pretreated with the indicated enzymes as described in Materials and Methods or were heated to 99 °C for 1 h. OK cells were then incubated with these extracts for 12 h before the uptake of ^{32}P -orthophosphate was measured over a 5-min period. The graph shows mean $\pm$ S.E.M. uptake of duplicate wells from two independent experiments. PTH: [Tyr34]hPTH(1–34)amide; PT: extract from parathyroid adenoma; A: extract from tumor A.

conditioned by primary cultures of an OOM tumor stimulated cAMP accumulation in UMR, but not in OK cells. Similarly, Miyauchi *et al.* (1988) found no cAMP response in primary renal cells when using an extract from a different tumor and Cai *et al.* (1994) were unable to detect cAMP accumulation in response to medium conditioned by tumor cells. In the present study, we found a cAMP-stimulatory activity in three of four tumor extracts, but only when using OK cells. Furthermore, for each of these three tumor extracts, the cAMP response correlated with the inhibition of ^{32}P uptake. In contrast, extract D, as well as extracts from two control tissues, kidney and liver, showed only little inhibition of ^{32}P uptake and failed to stimulate cAMP accumulation. However, the overall cAMP response was weak when compared with that induced by PTH, indicating that the inhibition of ^{32}P uptake may not be directly linked to the cAMP pathway.

Previous attempts to characterize the OOM factor chemically have yielded conflicting results. For example, based on dialysis experiments, one group suggested that the tumor-derived activity was larger than 8 kDa, but smaller than 25 kDa (Cai *et al.* 1994). Similar to our findings, a more recent preliminary report provided evidence for a low molecular weight entity that was resistant to several different proteinases, including trypsin and proteinase K (Nelson *et al.* 1999). Consistent with this latter finding, we found that tumor extract A could not be inactivated by various proteases. This could imply that the activity inhibiting phosphate transport is not a protein. In an attempt to partially purify the tumor-derived inhibitory factor(s), we obtained evidence for two different peaks of activity. First, we repeatedly and uniformly observed that all inhibitory activity was filtered through membranes with a cut-off of 3 kDa, suggesting a low molecular weight entity (note that synthetic PTH with a molecular weight

of 4.1 kDa was retained only partially by this filter). When fractionated by HPLC, the inhibitory activity was detected for all crude extracts in the flow-through (fraction no. 2), indicating that the C8 reverse-phase resin did not retain the factor. Extract A contained a second fraction with inhibitory activity, most likely explaining the higher efficacy of this extract. It is, however, possible that this second peak of inhibitory activity is present also in the other extracts, but in smaller quantities, making it necessary to fractionate larger amounts of tumor extract. The significant retention of inhibitory activity on the C8 reverse-phase resin may indicate that the biologically active factor is a small peptide with an amino acid sequence not recognized by the investigated enzymes or an amino acid derivative. In this regard, it is interesting to note that a fibroblast growth factor (FGF)-like molecule, FGF-23, was recently found to be mutated in patients with autosomal dominant hypophosphatemic rickets (White *et al.* 2000) and that the mRNA encoding this protein is expressed abundantly by all of the tumors described herein (White *et al.* 2001). It remains to be determined, however, whether FGF-23 or fragments thereof can induce phosphaturia *in vivo* or inhibit phosphate uptake in OK cells. Organic compounds with the ability to inhibit phosphate uptake, such as steroids or prostaglandins, cannot be ruled out but seem less likely because of the aqueous nature of the extracts. Furthermore, prostaglandin E₂, β -adrenergic agents and dopamine, a known inhibitor of ^{32}P uptake in OK cells (Baines & Drangova 1998), are not likely candidates, since the tumor extracts failed to stimulate cAMP accumulation in UMR106 and ROS 17/2.8 cells.

In conclusion, we have shown that low molecular weight molecules extracted from OOM tumors inhibit phosphate uptake in OK cells, but it remains uncertain

whether these are related to or derived from the long-sought phosphaturic factor that is thought to be produced by OOM tumors.

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