Accepted Manuscript

Phosphate toxicity and tumorigenesis

Ronald B. Brown, Mohammed S. Razzaque

PII: S0304-419X(18)30042-8
DOI: doi:10.1016/j.bbcan.2018.04.007
Reference: BBACAN 88218

To appear in:

Received date: 23 February 2018
Revised date: 17 April 2018
Accepted date: 18 April 2018

Please cite this article as: Ronald B. Brown, Mohammed S. Razzaque , Phosphate toxicity and tumorigenesis. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Bbacan(2018), doi:10.1016/j.bbcan.2018.04.007

Phosphate toxicity and tumorigenesis

Ronald B. Brown [1], and **Mohammed S. Razzaque** [2-4]

[1] School of Public Health and Health Systems, University of Waterloo, Waterloo, Ontario, Canada

[2] Department of Oral Health Policy & Epidemiology, Harvard School of Dental Medicine, Boston, MA, USA

[3] Department of Preventive & Community Dentistry, University of Rwanda College of Medicine & Health Sciences, School of Dentistry, Kigali, Rwanda

[4] Department of Pathology, Lake Erie College of Osteopathic Medicine, Erie, PA, USA

Keywords: diet, phosphate toxicity, tumorigenesis, klotho, FGF23,

Address of correspondence:

Mohammed S. Razzaque, MBBS, PhD, Department of Pathology, Lake Erie College of Osteopathic Medicine, 1858 West Grandview Boulevard, Room: B2-306, Erie, PA 16509, USA. E-mails: mrazzaque@lecom.edu; mrazzaque@hms.harvard.edu

Abstract

In this brief opinion article, we briefly summarized evidence that cellular phosphate burden from phosphate toxicity is a pathophysiological determinant of cancer cell growth. Tumor cells express more phosphate cotransporters and store more inorganic phosphate than normal cells, and dysregulated phosphate homeostasis is associated with the genesis of various human tumors. High dietary phosphate consumption causes the growth of lung and skin tumors in experimental animal models. Experimental studies show that excessive phosphate burden induces growth-promoting cell signaling, stimulates neovascularization, and is associated with chromosome instability and metastasis. Studies have also shown phosphate is a mitogenic factor that affects various tumor cell growth. Among epidemiological evidence linking phosphorus and tumor formation, the Health Professionals Follow-Up Study found that high dietary phosphorus levels were independently associated with lethal and high-grade prostate cancer. Further research is needed to determine how excessive dietary phosphate consumption influences initiation and promotion of tumorigenesis, and to elucidate prognostic benefits of reducing phosphate burden to decrease tumor cell growth and delay metastatic progression. The results of such studies could provide the basis for therapeutic modulation of phosphate metabolism for the improved patient outcome.

Introduction

The annual global burden of cancer is projected to increase to 21.6 million new cancer cases in the year 2030, a 53 percent increase from 2012 which is partially linked to increasing Westernization of lifestyle [1]. Also, increased life expectancy may also contribute to such a surge, as advanced age is an important risk factor for cancer development. Food and diet are considered important determinants of cancer, but the causal mechanisms are not fully understood [2]. Many substances identified as carcinogens in vitro do not always lead to tumor formation in vivo [3], implying that additional growth-promoting factors may be involved in tumorigenesis. In addition to excessive consumption of certain micronutrients that promote unwanted cellular replication, the possibility exists that reduced levels of certain factors and micronutrients, like magnesium, iron, zinc could either promote excessive cellular proliferation or facilitate DNA damage [4-6].

A hallmark of cancer is that cancer cells are self-sufficient in growth-promoting signals, enabling tumorigenesis to progress autonomously without exogenous stimuli [7]; yet, there is evidence that cancer growth is influenced by exogenous nutritional factors. In this brief opinion article, we will focus on the adverse effects of phosphate toxicity on tumorigenesis. Of clinical significance, after following cases for 24 years in the Health Professionals Follow-Up Study, high levels of dietary phosphorus were found to be independently associated with lethal and high-grade prostate cancer [8]. Milk and dairy products contribute the high percentage of phosphorus intake in the American diet [9], and Newmark and Heaney proposed that phosphate from dairy products is a risk factor correlated with prostate cancer in epidemiological studies of American male

physicians and Swedish males [10]. Three glasses of milk consumed a day compared to one glass increased cancer mortality in Swedish women by 44% (hazard ratio 1.44, 95% confidence interval 1.23 to 1.69) [11].

In addition, in a mouse model of hepatocellular carcinoma which was induced by the hepatitis B virus (HBV) transfection, hepatic tumor was formed in a dose-dependent manner by feeding these mice with higher concentrations of the milk protein casein [12]; the investigators also reported that mice fed with 6% dietary casein had approximately 75% fewer occurrences of tumors compared to mice fed with 22% dietary casein[12]. More importantly, when the reduced-casein dietary intervention was provided, even after tumor formation in these HBV-transfected mice, the intervention resulted in significant inhibition of tumor growth [12]. Similar observations were also noted in other studies; for instance, restricted casein feeding is linked to tumor growth inhibition in xenograft models of human breast and prostate cancer [13]. Of important relevance, casein is a phosphoprotein, rich in phosphorus; casein has been shown to increase the phosphorus content of laboratory rodent diets by as much as 50% [14].

In 1996, Schipper et al. proposed that cancer evolves from dysregulated metabolic control pathways rather than from autonomous growth of cells, and the researchers further proposed that this metabolic dysregulation process may be reversible [15]; for instance, eradication of Helicobacter pylori infection with reduced immunoinflammatory stimulus can lead to clinical and genetic reversibility of gastric lymphoma and carcinoma [16, 17]. Furthermore, the potential may exist to regulate cancer cell growth and progression by reversing the dysregulation of phosphate metabolism. In this brief opinion article, we present evidence to suggest that cellular

phosphate burden, associated with dysregulated phosphate metabolism, acts as a growth-promoting determinant in various human and experimental models of tumors. Some of the strongest evidence includes the role of excess dietary phosphate in stimulating cancer growth through cell signaling within the phosphoinositide 3-kinase (PI3K) pathway [18]. Other dcumentation includes the role of a phosphate-rich microenvironment in promoting cancer cell metastasis [19], clinical associations of elevated serum phosphate in certain cancer patients [20], and findings of abnormal amounts of phosphate retention in tumors [21].

Regulation of Phosphate Homeostasis

Phosphorus, an essential mineral, is ingested in food as natural phosphate compounds and chemical additives (polyphosphate, sodium phosphate, triphosphate, phosphoric acid, etc.) [9]. Organic phosphate compounds are digested to inorganic phosphate (Pi) in the form of dihydrogen phosphate (H_2PO_4) and mono-hydrogen phosphate (HPO_4). Serum phosphate levels are highly sensitive to regulation by hormones that facilitate multiorgan crosstalk among bones, kidneys, parathyroid glands, and intestine (**Figure 1**) [22-29]. Intestinal absorption of dietary phosphate intake is partly regulated by hormones such as bioactive vitamin D [$1,25(OH)_2D_3$], which increases active transport of phosphate by increasing intestinal expression of the type II sodium-phosphate co-transporter, NaPi2b. Serum phosphate levels are regulated primarily by the kidneys; most renal reabsorption of phosphate occurs in the proximal tubules of the kidneys through the expression of sodium-phosphate cotransporters NaPi2a and NaPi2c. Bone functions as a reserve for phosphate, and parathyroid hormone stimulates the release

of phosphorus from the hydroxyapatite bone matrix along with calcium during bone resorption. Bone also releases fibroblast growth factor 23 (FGF23), which is activated by its cofactor klotho to suppress renal phosphate transporters, decrease renal phosphate reabsorption, and increase urinary phosphate excretion [22-29]. FGF23 also suppresses bioactivation of vitamin D to decrease intestinal phosphate absorption and increase fecal phosphate excretion (**Figure 1**) [24, 30].

Inorganic phosphate is incorporated into cells as phospholipids, adenosine triphosphate (ATP), nucleic acids, and other metabolites necessary for cell growth, function, and survival [22]. Despite the indispensable role of phosphate in normal cellular functions, the molecular regulation of intra- and extra-cellular phosphorus metabolism is not yet clearly defined. It is however evident from clinical and experimental studies that when the bone-kidney-intestinal axis fails to regulate optimal circulatory levels, hyperphosphatemia persists, and cellular phosphate burden produces a deleterious effect on various tissues and organs, ranging from vascular calcification to premature aging to tumor formation [31-35].

Phosphate Transport and Tumorigenesis

The abnormal morphology of the tumor cell is characterized by loss of uniformity of the individual cells, defects in the architectural orientation of the differentiating cells, abnormal cytoplasm, an enlarged and irregularly shaped nucleus (increased nuclear-to-cytoplasmic ratios), and prominent nucleoli. Tumor cells stimulate vascular endothelial cell proliferation and form new capillaries through angiogenesis to support cancer cell growth, although the tumor neovascular network is unorganized, leaky, and sluggish

with an irregular flow [36]. Of biological importance, neovascularization is an important component of the tumor microenvironment that can influence tumor cell function, growth, and behavior such as cell-matrix interactions, possibly through lamellipodia and filopodia formation.

The membrane surface of cancer cells often contains filopodia and lamellipodia which provide adhesion and motility, sense the extracellular environment, and attract substrates [37, 38]. Of relevance, filopodia of osteoclast cells in bone seek out, break down, and transport calcium phosphate particles into the cell [39]; whether filopodia of soft tissue cells possess similar mineral ion transporting ability requires additional studies. Although biological behavior of mammalian cells and fungal cells are not always similar in response to an insult, it is however interesting to observe from a comparative biologic perspective that mycorrhiza fungi cells from a plant root system have hyphae that extend into the soil and are equipped with inorganic phosphate transporters (PiT) for cross-membrane intake and retention of orthophosphate such as H_2PO_4 [40, 41]. Evidence shows that additional cross-membrane transporters allow cancer cells to absorb greater amounts of phosphate from the tumor microenvironment. For example, ovarian carcinomas were found to overexpress sodium-phosphate co-transporter NaPi2b, encoded by the SLC34A2 gene [42]. Increased expression of NaPi2b, more so than in normal tissue, was also found in thyroid, breast, and lung cancer [43], and fungal hyphae are prevalent in biopsies of precancerous epithelial lesions [44]. For instance, epithelial dysplasia and prevalence of fungal hyphae were significantly higher in leukoplakia (precancerous oral lesion) as compared to lichen planus and submucous oral fibrosis; higher grades of dysplasia were associated with

increased prevalence of fungal hyphae [44]. Of clinical importance, the presence of fungal hyphae in potentially malignant lesions may be a useful indicator in predicting malignant transformation [44]. Furthermore, studies need to be conducted to determine whether phosphate transporter expression in tumor cells is limited to a certain carcinoma or it is a generalized phenomenon that may be present in sarcoma and other non-solid tumor cells, as well.

The pointy, spiculated masses of some tumors were named “cancer” by Hippocrates, which described the tumors’ crab-leg shape [45-47]. Certain tumor cells extend into and move throughout the interstitium, equipped with overexpressed NaPi2b transporters capable of absorbing high levels of phosphorus from the tumor microenvironment. Inactivating the function of NaPi2b in the lung resulted in suppressed tumor growth, reduced cell proliferation and increased apoptotic cell death in a K-ras mutated murine model of lung cancer [48]. Furthermore, angiogenesis and neovascularization in breast cancer and lung cancer cells, in combination with human umbilical vascular endothelial cells, were stimulated in vitro by a high-phosphorus microenvironment [49]. High phosphate concentration increased gene expression of forkhead box protein C2 (FOXC2), osteopontin, and vascular endothelial growth factor in a dose-dependent manner; endothelial cell migration and tube formation were also observed in the high-phosphate microenvironment [49]; from the in vitro observations, the authors proposed that limiting the availability of phosphorus through reduced consumption could impair the growth of the ‘more metabolically active’ tumor cells while preserving ‘less metabolically active’ healthy cells [49].

Phosphate Sequestration and Tumorigenesis

Inorganic phosphate, unbound to organic complexes, is mostly found in extracellular fluids, including the serum. Tumorigenesis develops in tissue containing cells with storage properties capable of supporting sequestration; tumors sequester more phosphorus isotope P32 than healthy tissue, which is why this radioactive isotope came into use in the early 20th century for tumor detection. Patients with advanced stages of malignant tumors were injected with P32 and examined after death; malignant cells within deceased patients were found to retain concentrations of P32 equal to or greater than concentrations in other highly metabolic tissue [50]. Malignant skin tumors also absorbed more P32 than benign tumors [51], greater concentrations of P32 were absorbed in leukemic tissue [52, 53], and mammary carcinoma, lymphoma, and lymphosarcoma tissue had a higher and faster uptake of P32 with longer retention than in normal tissue [54].

Towards the end of the 20th century, magnetic resonance spectroscopy studies detected numerous organic phosphorus metabolites in tumor cells [55]. Of relevance, the amount of inorganic phosphate found in human cancer cells (HeLa cells) exceeded the phosphorus content of any of the organic phosphorus metabolites [55], verifying sequestration of excess inorganic phosphate in cancer cells. Compared to normal cells, up to twice as much inorganic phosphate was detected in cancer cells of the colon and lungs [21]. More recently a two-fold increase in inorganic phosphate concentration within the interstitial microenvironment of tumors was confirmed through in vivo assessment using advanced electron paramagnetic resonance profiling [19]. High phosphorus content within the fluid of type-1 cysts in humans is also associated with

increased breast cancer risk [56], and high levels of signalling phosphoprotein expression were found in ovarian tumors [57]. In addition to phosphate sequestration and tumorigenesis, hyperphosphatemia can directly activate certain gene expression through ERK1/2 and AKT signaling in epithelial and mesenchymal cells (e.g. fibroblasts), inducing epithelial-mesenchymal transformation and synthesis of matrix proteins [58, 59]; of particular importance, both epithelial-mesenchymal transformation and matrix production are important events for tumor growth and development. Additionally, hyperphosphatemia in animal models was shown to upregulate endothelin-1 in endothelial cells, which increases reactive oxygen species and creates oxidative stress associated with inflammation, aging, and cancer [60]. Moreover, hyperphosphatemia can directly damage the endothelial cells by disrupting the mitochondrial membrane, generating reactive oxygen species, reducing nitric oxide production and inducing apoptosis, partly by activating the PKC pathway [61, 62], some of these events are also involved in tumorigenesis.

Phosphate Toxicity and Tumorigenesis

Cancer patients were observed to have over twice the mean value of phosphorus in their blood compared to patients without cancer: 7.80 ($\pm$2.24) mg/dL and 3.38 ($\pm$0.58) mg/dL, respectively ($P<0.001$) [20]. The researchers attributed elevated extracellular phosphate levels to the increased metabolic activity in cancer cells. In an examination of 397,292 participants from the Swedish AMORIS study, Wulaningsih et al. found that high serum phosphorus levels were positively correlated with risk for cancers of the lung, pancreas, thyroid, and bone in men, and cancers of the esophagus, lung, and

nonmelanoma skin cancer in women. However, high serum phosphate levels were negatively correlated with risk for breast and endometrial cancer in women, which the researchers suggested “reflects underlying estrogen levels” [63]. It is noted that estrogen is a mitogen that causes rapid cell division in reproductive tissue, and high serum phosphate levels are normally associated with rapid growth, as observed in infants and children. It should be mentioned that estrogen can suppress the expression of phosphate transporter, NaPi2a, in the kidneys, and enhance the production of FGF23 in the bones [64]. However, this effect is related to estrogen treatment and may be influenced by other pharmacological factors. Nevertheless, the interplay between estrogen and phosphate levels as a contributor to tumor progression requires further carefully designed studies.

Elevated serum phosphate levels at first diagnosis of lung cancer predicted patients’ disease stage and affected patient survival [65]. In a retrospective clinic-based cohort study of 1,241 colorectal cancer patients, post-operative hyperphosphatemia was negatively associated with patient survival [66]. In a similar line of observation, a study conducted on 110 patients with multiple myeloma found that high serum phosphate levels were significantly associated with shorter survival [67]. An unexpected finding in people who use heroin regularly had a normal serum phosphorus level compared to healthy subjects, and a retrospective analysis of 2,321 deceased heroin addicts from 2001 to 2010, not a single recorded death from cancer was detected [68]. Of relevance, heroin users have dysfunctional eating patterns, eat infrequently, ingest inadequate amounts of food, consume low levels of proteins, and derive much of their calories from sugary sweets [69]. This dietary pattern may contribute to low intake of dietary

phosphorus and high renal phosphate reabsorption to maintain normal serum phosphate levels; although this hypothesis needs further experimental validation, as direct cause-and-effect relation is not yet established.

A ketogenic diet was effective in reducing tumors in animal experiments [70], and the diet reduced progression of gliomas in brain cancer patients [71]. An analysis of the micronutrient content of a classic high-fat, low-carbohydrate ketogenic diet for children shows that the diet is low in phosphorus. For example, a sample ketogenic menu [72], modified in amounts for an adult, provides 2025 calories a day with a 4:1 ratio of fat to non-fat grams and only 599 mg of phosphorus, which is below the adult RDI of 700 mg. The low phosphate content of the classic ketogenic diet may be a determinant in reducing the size and progression of tumors, and further experimental investigations are warranted.

Phosphate Dysregulation and Cancer Cell Signaling

During stages of dysplasia and hyperplasia that may lead to cancer, atypical cells develop enlarged, irregularly shaped nucleoli that can upregulate ribosome biogenesis and increase production of ribosomal RNA (rRNA) [73]. The complex molecular machinery of the ribosome is composed of several distinct proteins and nucleic acids, and is vital for protein synthesis in living and proliferating cells. Rich in phosphorus, rRNA supports growth in rapidly dividing cells through protein biosynthesis, which is consistent with the tumor growth rate hypothesis [74]. Noting that phosphorus is a limiting factor in biological growth rate, as well as being the least abundantly supplied element that forms nucleic acids DNA and RNA, Elser et al. published a mathematical

calculation based on the growth rate hypothesis which predicted that reducing the tumor's phosphorus intake in half will reduce three-quarters of the tumor size [75]. In an earlier study from 1955, phosphorus was found to play a key role in promoting precancerous tissue to cancer through increased phosphorus uptake into nuclear RNA, thus increasing cancer cell proliferation [76]. Of significant importance, the researchers found that carcinogenesis in precancerous tissue was delayed when phosphorus uptake by nuclear RNA was suppressed [76].

Similar to cytokines and various growth factors, phosphate can also induce cancer cell growth through various growth promoting signaling, including the PI3K pathway [18] (**Figure 2**); PI3K phosphorylates Akt (protein kinase B) to activate mTOR kinase, which in turn suppresses cell apoptosis and up-regulates synthesis of protein by phosphorylating important regulators of mRNA translation [77]. Of particular relevance, Jin et al. found that high dietary phosphate activated Akt phosphorylation, a key regulator of tumor growth, thus facilitating cap-dependent protein translation and increasing lung tumorigenesis in mice [78]. The researchers also found that high dietary phosphate suppressed phosphatase and tensin homolog (PTEN), a tumor suppressor phosphatase, and inorganic phosphate suppressed carboxyl-terminal modulator protein (CTMP), a negative regulator of Akt activity. Another signaling pathway associated with tumor growth is regulated by the GTP-binding protein N-ras. Camalier et al. found that a high-phosphorus diet activated N-ras and increased skin papilloma by around 50% in experimental animals [79].

Evidence also shows an association between high phosphate concentration and chromosome instability, another hallmark of cancer. Hyperphosphatemia, induced by

endocrine dysregulation, increased the risk of chromosome aberrations in the parathyroid glands of patients with hyperparathyroidism [80], and genotoxic effects from exposure to phosphate food additives were induced in lymphocytes [81]. Additionally, DNA damage is associated with chronic renal failure in patients, within whom phosphate metabolism is often dysregulated [82].

Tumor cells contain self-digesting lysosomal proteases that are active during the catabolic process of autophagy and apoptosis; more so than in normal tissue [83]. These proteases interact with and modulate phosphorylating kinases in signaling pathways to lower cell proliferation in cancer [84]. Of relevance, high dietary phosphate, shown to increase phosphorylation of kinases in the Akt pathway of tumorigenesis [78], may increase tumorigenesis through the predominance of phosphorylating kinases over modulating proteases. This is further evidenced by apoptosis suppression after increased kinase phosphorylation during cross-talk among the signaling pathways [85, 86]. The balance between phosphorylating kinases and modulating proteases helps explain why lower phosphorus diets, associated with fewer phosphorylating kinases, may increase the preponderance of proteases that reduce cancer cell proliferation. How such interaction between kinases and proteases may be influenced by the total body phosphate content needs additional studies.

Recent evidence has shown that phosphate starvation is sufficient to induce autophagy in eukaryotic yeast cells (Saccharomyces cerevisiae) [87]. The researchers found that phosphate starvation resulted in dephosphorylation of autophagy protein Atg13 which initiates the autophagy process. Whether such phenomenon in the yeast can be relevant to the mammalian system needs experimental validation; it is, however,

important to mention that Atg11 was also a necessary factor in phosphate starvation-induced autophagy [87]. It is noteworthy that Atg 13 dephosphorylation is caused by inactivation of the TORC1 signaling pathway; a pathway which researchers have shown is responsive to phosphorus levels in cancer cells [87].

Phosphate and Tumor Metastasis

The “seed and soil” hypothesis of metastasis proposes that tumors spread through wandering rogue cancer cells in the blood and lymph; yet, corroboration of this and related hypotheses of metastasis are not always easy to demonstrate in an in vivo setup, and are beyond current technological capabilities [88]. Alternatively, Ramirez and Fiedler reported that a high concentration of inorganic phosphate plays a role in tumor cell behavior [89], and it is hypothesized that phosphate and perhaps associated factors released from bone during osteolysis could produce a microenvironment that facilitates bone metastasis in breast tumors by modulating the activity of cancer cells. Recently, researchers using in vivo electron paramagnetic resonance profiling with a specially developed probe found that interstitial inorganic phosphate concentration within the tumor microenvironment provided a marker that can distinguish highly metastatic tumors from non-metastatic tumors [19]. This is the first in vivo evidence suggesting that systemic phosphate toxicity may be an important determinant of metastasis. The pathobiological plausibility of this determinant is further supported by evidence of conditions linked to phosphate toxicity in cancer patients, discussed below.

Dysregulation of phosphate metabolism is associated with conditions such as chronic kidney disease-mineral and bone disorder (CKD-MBD) and ectopic calcification,

including vascular calcification, which are often present as comorbidities in cancer patients. For example, various types of cancer is closely associated with CKD [90], bone disorders [91], and cardiovascular diseases [92]. CKD patients receiving dialysis to control hyperphosphatemia have a higher rate of comorbid cancers than the general population, including cancers of the bladder, kidney, liver, thyroid, tongue, cervix, as well as non-Hodgkin lymphoma and multiple myeloma [93]. Of relevance, paraneoplastic nephropathies are well-documented complications for numerous tumors, including lung tumors, gastrointestinal tumors and Hodgkin's disease [94, 95]. Moreover, the risk of epileptic seizures is significantly increased in cancer patients [96], and seizures occur in tumor lysis syndrome as excess phosphorus is released into the serum and causes secondary hypocalcemia [97]. Toxic conditions related to dysregulated phosphate metabolism, including malnutrition and systemic inflammation [98], are also seen in cachexia of terminal cancer patients [99]. As mentioned, patients with end-stage CKD receiving either dialysis or transplantation are at higher risk for developing a wide range of tumors, including of the lung and prostate [100-102]. More concerning is the documentation of increased risk for cancer in CKD patients, even at the early stages of the disease [103, 104]. Of clinical significance, phosphate toxicity is a major disease manifestation noted in patients with all stages of CKD; how phosphate toxicity promotes tumorigenesis and eventual metastasis in patients with CKD needs further clinical and experimental studies.

The enzyme alpha-klotho is an endocrine regulatory factor in phosphate homeostasis that has been linked to tumor suppression [105, 106]. Klotho acts as a cofactor with FGF23 that decreases renal reabsorption of inorganic phosphate [107].

The FGF23-klotho complex suppresses the expression and function of Na^+-dependent phosphate co-transporters NaPi2a and NaPi2c in the renal tubules, thus reducing renal reabsorption and increasing phosphaturia. Klotho expression is reported to be reduced in many types of tumor tissues compared to corresponding normal tissues, including breast, pancreatic, liver, cervical, ovary, gastric, prostate, esophageal, rectal, colon, melanoma, and some lung cancer types [108]. Decreased klotho expression may be related to increased phosphate levels as clearly shown in earlier mouse genetic studies [34, 109-114], and in patients with a mutation in the Klotho gene [115]. In vitro studies also suggest that Klotho inhibits the Wnt/B-catenin pathway in human hepatocellular carcinoma [116], and the extracellular KL1 domain of the mouse Klotho protein, rather than the K2 domain, was found to actively suppress breast cancer cells [117]. From recently reported evidence, it appears more likely that the essential phosphate-regulating factor klotho is a tumor suppressor gene, as well [118]. The expression of Klotho gene is shown to be suppressed by hypermethylation of DNA in various cancer cells, including cervical, colorectal, gastric and breast cancers [119-121], implicating that expression of Klotho may be closely associated with genesis of tumor in certain tissues and organs. As mentioned, Klotho can suppress cell proliferation and metastasis by inhibiting growth factor signaling such as insulin-like growth factor 1 (IGF-1) and transforming growth factor beta (TGF-β) [118, 122].

Conclusion

In this brief opinion article, we summarized the evidence showing that experimental animals fed high-phosphate diets increased the risk of cancer growth relative to diets

with lower levels of phosphate. Pathophysiological evidence indicates that tumors sequester excess levels of serum phosphorus while stimulating tumorigenesis, neovascularization, chromosome instability, and possibly metastasis (**Figure 3**). These findings are supported by additional clinical and epidemiological associations between high levels of dietary phosphate consumption, increased serum phosphate levels, tumorigenesis, and comorbidities in cancer patients. Of clinical importance, phosphate toxicity could be both cause (tumor-promoting factor) and consequences (cachexia) of tumorigenesis. Finally, we propose that cancer patients may receive therapeutic benefits upon referral to renal dietitians who are skilled in restricting patients' dietary phosphorus intake, and we encourage further research of dietary phosphate manipulation in the initiation, promotion, progression, recovery, and prevention of cancer cell growth.

Acknowledgments

Thanks to Dr. John Mielke, Mr. M. Muhit Razzaque and Mrs. Rufsa H. Afroze for carefully reading the manuscript and providing useful suggestions. Dr. Razzaque is a Visiting Professor at the Harvard School of Dental Medicine, Boston (USA) and an Honorary Professor at the University of Rwanda College of Medicine & Health Sciences in Kigali (Rwanda).

Figure-1

Total body phosphorus homeostasis is primarily maintained by a multi-organ cross-talk among parathyroid gland, intestine, kidney, and bone; intestinal absorption of dietary phosphate, renal reabsorption of ultrafiltrated phosphate in proximal tubular epithelial cells, and the shift of intracellular phosphate between extracellular and storage phosphate determine the phosphate homeostasis. Of clinical importance, since less than 1% of total body phosphorus is extracellular, the serum phosphorus concentration does not truly reflect total body phosphorus content and is also a poor predictor of intracellular (~15%) and storage (~85%) phosphorus content [22].

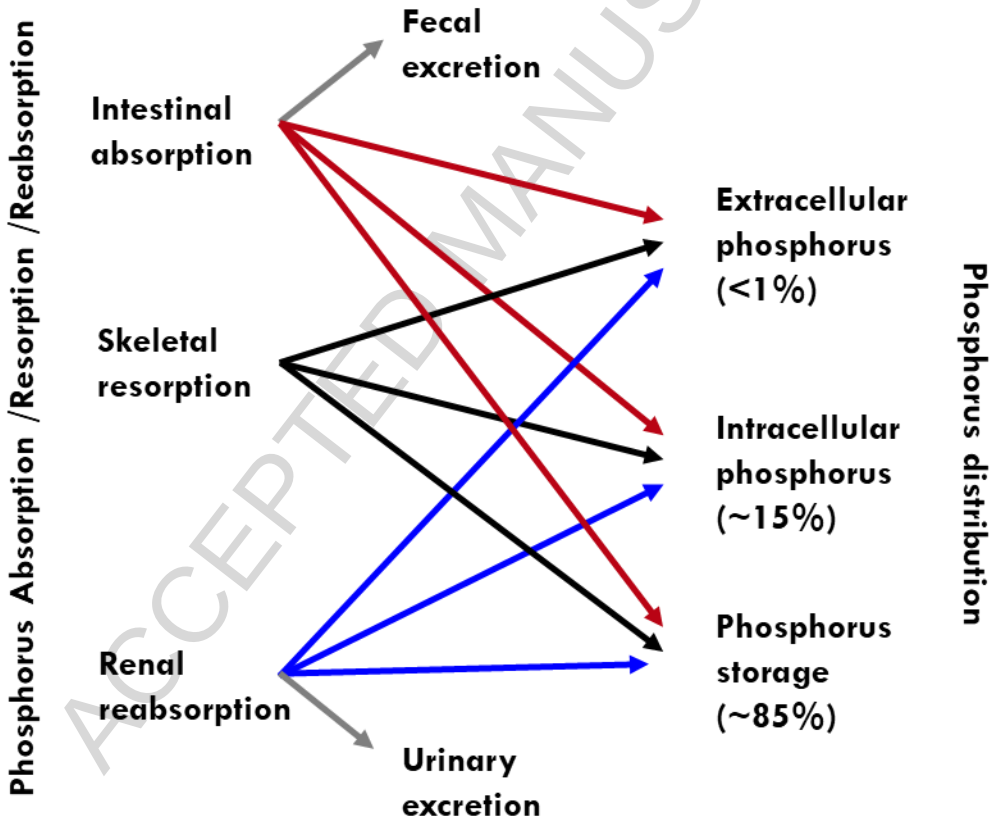


Figure-2

Cancer cells respond to growth factors through a signaling pathway in which phosphoinositide 3-kinase (PI3K) phosphorylates Akt (protein kinase B) [18]. Akt activates mTOR kinase, which in turn can suppress apoptotic cell removal and induce cell proliferation [77]. High dietary phosphate was found to activate Akt phosphorylation, facilitating cap-dependent protein translation and increasing lung tumorigenesis in mice [78]. High dietary phosphate in mice also suppressed PTEN, a tumor suppressor phosphatase, and suppressed CTMP, a negative regulator of Akt activity [78].

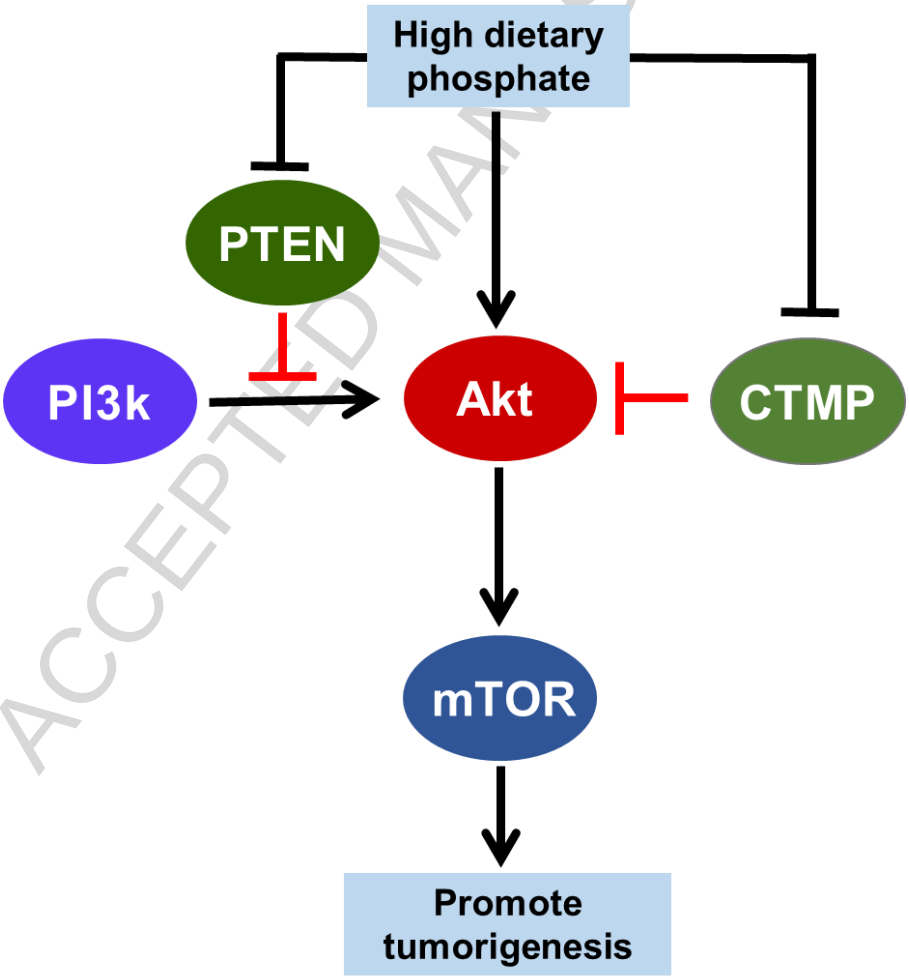
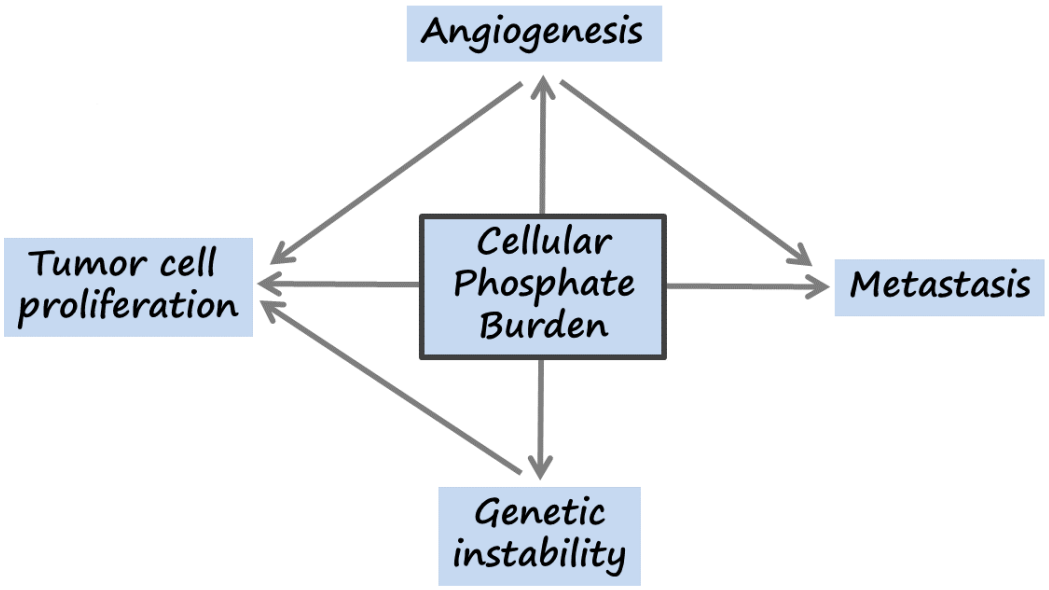


Figure-3

Studies have shown that cellular phosphate burden can stimulate tumorigenesis [76], possibly by exerting mitogenic effects on tumor cells, promoting angiogenesis [49], inducing chromosome instability [80], and facilitating metastasis [19].



References

[1] F. Bray, A. Jemal, N. Grey, J. Ferlay, D. Forman, Global cancer transitions according to the Human Development Index (2008-2030): a population-based study, Lancet Oncol, 13 (2012) 790-801.

[2] V.L. Go, D.A. Wong, Y. Wang, R.R. Butrum, H.A. Norman, L. Wilkerson, Diet and cancer prevention: evidence-based medicine to genomic medicine, J Nutr, 134 (2004) 3513S-3516S.

[3] cancer.org, Known and probable human carcinogens, American Cancer Society, 2015.

[4] A.M. Uwitonze, M.S. Razzaque, Role of Magnesium in Vitamin D Activation and Function, J Am Osteopath Assoc, 118 (2018) 181-189.

[5] E. Ho, Zinc deficiency, DNA damage and cancer risk, J Nutr Biochem, 15 (2004) 572-578.

[6] N. Hung, C.C. Shen, Y.W. Hu, L.Y. Hu, C.M. Yeh, C.J. Teng, A.S. Kuan, S.C. Chen, T.J. Chen, C.J. Liu, Risk of cancer in patients with iron deficiency anemia: a nationwide population-based study, PLoS One, 10 (2015) e0119647.

[7] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell, 144 (2011) 646-674.

[8] K.M. Wilson, I.M. Shui, L.A. Mucci, E. Giovannucci, Calcium and phosphorus intake and prostate cancer risk: a 24-y follow-up study, Am J Clin Nutr, 101 (2015) 173-183.

[9] M.S. Calvo, A.J. Moshfegh, K.L. Tucker, Assessing the health impact of phosphorus in the food supply: issues and considerations, Adv Nutr, 5 (2014) 104-113.

[10] H.L. Newmark, R.P. Heaney, Dairy products and prostate cancer risk, Nutr Cancer, 62 (2010) 297-299.

[11] K. Michaelsson, A. Wolk, S. Langenskiold, S. Basu, E. Warensjo Lemming, H. Melhus, L. Byberg, Milk intake and risk of mortality and fractures in women and men: cohort studies, BMJ, 349 (2014) g6015.

[12] Z. Cheng, J. Hu, J. King, G. Jay, T.C. Campbell, Inhibition of hepatocellular carcinoma development in hepatitis B virus transfected mice by low dietary casein, Hepatology, 26 (1997) 1351-1354.

[13] L. Fontana, R.M. Adelaiye, A.L. Rastelli, K.M. Miles, E. Ciamporcero, V.D. Longo, H. Nguyen, R. Vessella, R. Pili, Dietary protein restriction inhibits tumor growth in human xenograft models, Oncotarget, 4 (2013) 2451-2461.

[14] P.G. Reeves, F.H. Nielsen, G.C. Fahey, Jr., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, J Nutr, 123 (1993) 1939-1951.

[15] H. Schipper, E.A. Turley, M. Baum, A new biological framework for cancer research, Lancet, 348 (1996) 1149-1151.

[16] M.I. Pereira, J.A. Medeiros, Role of Helicobacter pylori in gastric mucosa-associated lymphoid tissue lymphomas, World J Gastroenterol, 20 (2014) 684-698.

[17] C.Y. Wu, K.N. Kuo, M.S. Wu, Y.J. Chen, C.B. Wang, J.T. Lin, Early Helicobacter pylori eradication decreases risk of gastric cancer in patients with peptic ulcer disease, Gastroenterology, 137 (2009) 1641-1648 e1641-1642.

[18] P. Liu, H. Cheng, T.M. Roberts, J.J. Zhao, Targeting the phosphoinositide 3-kinase pathway in cancer, Nat Rev Drug Discov, 8 (2009) 627-644.

[19] A.A. Bobko, T.D. Eubank, B. Driesschaert, I. Dhimitruka, J. Evans, R. Mohammad, E.E. Tchekneva, M.M. Dikov, V.V. Khramtsov, Interstitial Inorganic Phosphate as a Tumor Microenvironment Marker for Tumor Progression, Sci Rep, 7 (2017) 41233.

[20] C.D. Papaloucas, M.D. Papaloucas, V. Kouloulias, K. Neanidis, K. Pistevou-Gompaki, J. Kouvaris, A. Zygogianni, K. Mystakidou, A.C. Papaloucas, Measurement of blood phosphorus: a quick and inexpensive method for detection of the existence of cancer in the body. Too good to be true, or forgotten knowledge of the past?, Med Hypotheses, 82 (2014) 24-25.

[21] J.J. Elser, M.M. Kyle, M.S. Smith, J.D. Nagy, Biological stoichiometry in human cancer, PLoS One, 2 (2007) e1028.

[22] R.B. Brown, M.S. Razzaque, Endocrine regulation of phosphate homeostasis, Textbook of Nephro-Endocrinology, 2nd edition, Elsevier, London, United Kingdom, 2018, pp. 539-548.

[23] M.S. Razzaque, Bone-kidney axis in systemic phosphate turnover, Arch Biochem Biophys, 561 (2014) 154-158.

[24] M.S. Razzaque, The FGF23-Klotho axis: endocrine regulation of phosphate homeostasis, Nat Rev Endocrinol, 5 (2009) 611-619.

[25] M.S. Razzaque, FGF23-mediated regulation of systemic phosphate homeostasis: is Klotho an essential player?, Am J Physiol Renal Physiol, 296 (2009) F470-476.

[26] R.G. Erben, O. Andrukhova, FGF23-Klotho signaling axis in the kidney, Bone, 100 (2017) 62-68.

[27] S. Kinoshita, M. Kawai, The FGF23/KLOTHO Regulatory Network and Its Roles in Human Disorders, Vitam Horm, 101 (2016) 151-174.

[28] M.S. Razzaque, B. Lanske, The emerging role of the fibroblast growth factor-23-klotho axis in renal regulation of phosphate homeostasis, J Endocrinol, 194 (2007) 1-10.

[29] C.P. Kovesdy, L.D. Quarles, FGF23 from bench to bedside, Am J Physiol Renal Physiol, 310 (2016) F1168-1174.

[30] R.B. Brown, A. Haq, C.F. Stanford, M.S. Razzaque, Vitamin D, phosphate, and vasculotoxicity, Can J Physiol Pharmacol, 93 (2015) 1077-1082.

[31] J.M. Goodson, P. Shi, C.H. Mumena, A. Haq, M.S. Razzaque, Dietary phosphorus burden increases cariogenesis independent of vitamin D uptake, J Steroid Biochem Mol Biol, 167 (2017) 33-38.

[32] R.B. Brown, M.S. Razzaque, Phosphate toxicity: a stealth biochemical stress factor?, Med Mol Morphol, 49 (2016) 1-4.

[33] S. Osuka, M.S. Razzaque, Can features of phosphate toxicity appear in normophosphatemia?, J Bone Miner Metab, 30 (2012) 10-18.

[34] M. Ohnishi, S. Kato, J. Akiyoshi, A. Atfi, M.S. Razzaque, Dietary and genetic evidence for enhancing glucose metabolism and reducing obesity by inhibiting klotho functions, FASEB J, 25 (2011) 2031-2039.

[35] M.S. Razzaque, Phosphate toxicity: new insights into an old problem, Clin Sci (Lond), 120 (2011) 91-97.

[36] A.I. Baba, C. Câtoi, Tumor cell morphology, Comparative oncology, Romanian Academy, Place Published, 2007.

[37] G. Jacquemet, H. Hamidi, J. Ivaska, Filopodia in cell adhesion, 3D migration and cancer cell invasion, Curr Opin Cell Biol, 36 (2015) 23-31.

[38] A.K. Guo, Y.Y. Hou, H. Hirata, S. Yamauchi, A.K. Yip, K.H. Chiam, N. Tanaka, Y. Sawada, K. Kawauchi, Loss of p53 enhances NF-kappaB-dependent lamellipodia formation, J Cell Physiol, 229 (2014) 696-704.

[39] T. Nagafusa, H. Hoshino, T. Sakurai, S. Terakawa, A. Nagano, Mechanical fragmentation and transportation of calcium phosphate substrate by filopodia and lamellipodia in a mature osteoclast, Cell Biol Int, 31 (2007) 1150-1159.

[40] M. Willmann, N. Gerlach, B. Buer, A. Polatajko, R. Nagy, E. Koebke, J. Jansa, R. Flisch, M. Bucher, Mycorrhizal phosphate uptake pathway in maize: vital for growth and cob development on nutrient poor agricultural and greenhouse soils, Front Plant Sci, 4 (2013) 533.

[41] C. Rausch, P. Daram, S. Brunner, J. Jansa, M. Laloi, G. Leggewie, N. Amrhein, M. Bucher, A phosphate transporter expressed in arbuscule-containing cells in potato, Nature, 414 (2001) 462-470.

[42] K. Levan, M. Mehryar, C. Mateoiu, P. Albertsson, T. Back, K. Sundfeldt, Immunohistochemical evaluation of epithelial ovarian carcinomas identifies three different expression patterns of the MX35 antigen, NaPi2b, BMC Cancer, 17 (2017) 303.

[43] M. D'Arcangelo, O.T. Brustugun, Y. Xiao, Y. Choi, C. Behrens, L.M. Solis, Y. Wang, R. Firestein, T.A. Boyle, M. Lund-Iversen, Prevalence and prognostic significance of sodium-dependent phosphate transporter 2b (NaPi2b) protein expression in non-small cell lung cancer (NSCLC), Annal Oncol, 25 (2014) iv66-iv67.

[44] B.P. Hongal, V.V. Kulkarni, R.S. Deshmukh, P.S. Joshi, P.P. Karande, A.S. Shroff, Prevalence of fungal hyphae in potentially malignant lesions and conditions-does its occurrence play a role in epithelial dysplasia?, J Oral Maxillofac Pathol, 19 (2015) 10-17.

[45] S.I. Hajdu, A note from history: landmarks in history of cancer, part 3, Cancer, 118 (2012) 1155-1168.

[46] S.I. Hajdu, A note from history: landmarks in history of cancer, part 2, Cancer, 117 (2011) 2811-2820.

[47] S.I. Hajdu, A note from history: landmarks in history of cancer, part 1, Cancer, 117 (2011) 1097-1102.

[48] S.H. Hong, A. Minai-Tehrani, S.H. Chang, H.L. Jiang, S. Lee, A.Y. Lee, H.W. Seo, C. Chae, G.R. Beck, Jr., M.H. Cho, Knockdown of the sodium-dependent phosphate co-transporter 2b (NPT2b) suppresses lung tumorigenesis, PLoS One, 8 (2013) e77121.

[49] Y. Lin, K.E. McKinnon, S.W. Ha, G.R. Beck, Jr., Inorganic phosphate induces cancer cell mediated angiogenesis dependent on forkhead box protein C2 (FOXC2) regulated osteopontin expression, Mol Carcinog, (2014).

[50] L.A. Erf, J.H. Lawrence, Phosphorus Metabolism in Neoplastic Tissue, Proc Soc Exp Biol Med 46 (1941) 694-695.

[51] F.K. Bauer, C.G. Steffen, Radioactive phosphorus in the diagnosis of skin tumors; differentiation of nevi, malignant melanomas, and other skin tumors, J Am Med Assoc, 158 (1955) 563-565.

[52] S. Warren, The retention of radioactive phosphorus in leukemic patients, Cancer Res, 3 (1943) 872-876.

[53] N.I. Berlin, Treatment of the myeloproliferative disorders with 32P, Eur J Haematol, 65 (2000) 1-7.

[54] H.B. Jones, I.L. Chaikoff, J.H. Lawrence, Phosphorus Metabolism of Neoplastic Tissues (Mammary Carcinoma, Lymphoma, Lymphosarcoma) as Indicated by Radioactive Phosphorus, Am J Cancer, 40 (1940) 243-250.

[55] F.E. Evans, N.O. Kaplan, 31P nuclear magnetic resonance studies of HeLa cells, Proc Natl Acad Sci U S A, 74 (1977) 4909-4913.

[56] A.R. Sisman, B. Sis, T. Canda, B. Onvural, Electrolytes and trace elements in human breast cyst fluid, Biol Trace Elem Res, 128 (2009) 18-30.

[57] D. Faratian, I. Um, D.S. Wilson, P. Mullen, S.P. Langdon, D.J. Harrison, Phosphoprotein pathway profiling of ovarian carcinoma for the identification of potential new targets for therapy, Eur J Cancer, 47 (2011) 1420-1431.

[58] Z. Chen, D. Chen, T.L. McCarthy, M. Centrella, Y. Zhang, G.W. Moeckel, Inorganic phosphate stimulates fibronectin expression in renal fibroblasts, Cell Physiol Biochem, 30 (2012) 151-159.

[59] G.R. Beck, Jr., B. Zerler, E. Moran, Phosphate is a specific signal for induction of osteopontin gene expression, Proc Natl Acad Sci U S A, 97 (2000) 8352-8357.

[60] G. Olmos, P. Martinez-Miguel, E. Alcalde-Estevez, D. Medrano, P. Sosa, L. Rodriguez-Manas, M. Naves-Diaz, D. Rodriguez-Puyol, M.P. Ruiz-Torres, S. Lopez-Ongil, Hyperphosphatemia induces senescence in human endothelial cells by increasing endothelin-1 production, Aging Cell, 16 (2017) 1300-1312.

[61] G.S. Di Marco, M. Hausberg, U. Hillebrand, P. Rustemeyer, W. Wittkowski, D. Lang, H. Pavenstadt, Increased inorganic phosphate induces human endothelial cell apoptosis in vitro, Am J Physiol Renal Physiol, 294 (2008) F1381-1387.

[62] E. Shuto, Y. Taketani, R. Tanaka, N. Harada, M. Isshiki, M. Sato, K. Nashiki, K. Amo, H. Yamamoto, Y. Higashi, Y. Nakaya, E. Takeda, Dietary phosphorus acutely impairs endothelial function, J Am Soc Nephrol, 20 (2009) 1504-1512.

[63] W. Wulaningsih, K. Michaelsson, H. Garmo, N. Hammar, I. Jungner, G. Walldius, L. Holmberg, M. Van Hemelrijck, Inorganic phosphate and the risk of cancer in the Swedish AMORIS study, BMC Cancer, 13 (2013).

[64] S. Faroqui, M. Levi, M. Soleimani, H. Amlal, Estrogen downregulates the proximal tubule type IIa sodium phosphate cotransporter causing phosphate wasting and hypophosphatemia, Kidney Int, 73 (2008) 1141-1150.

[65] V. Kouloulias, M. Tolia, N. Tsoukalas, C. Papaloucas, K. Pistevou-Gombaki, A. Zygogianni, K. Mystakidou, J. Kouvaris, M. Papaloucas, A. Psyrri, G. Kyrgias, V. Gennimata, K. Leventakos, I. Panayiotides, Z. Liakouli, N. Kelekis, A. Papaloucas, Is there any potential clinical impact of serum phosphorus and magnesium in patients with lung cancer at first diagnosis? A multi-institutional study, Asian Pac J Cancer Prev, 16 (2015) 77-81.

[66] Z. Ye, J.P. Palazzo, L. Lin, Y. Lai, F. Guiles, R.E. Myers, J. Han, J. Xing, H. Yang, Postoperative hyperphosphatemia significantly associates with adverse survival in colorectal cancer patients, J Gastroenterol Hepatol, 28 (2013) 1469-1475.

[67] M. Umeda, S. Okuda, H. Izumi, D. Nagase, Y. Fujimoto, Y. Sugasawa, C. Arai, K. Natori, M. Katoh, Y. Kuraishi, Prognostic significance of the serum phosphorus level and its relationship with other prognostic factors in multiple myeloma, Ann Hematol, 85 (2006) 469-473.

[68] V. Kouloulias, C.D. Papaloucas, A. Papaloucas, What is the connection between serum phosphorus, cancer and heroin? A medical hypothesis based on observational studies, J BUON, 22 (2017) 1303-1306.

[69] J. Neale, S. Nettleton, L. Pickering, J. Fischer, Eating patterns among heroin users: a qualitative study with implications for nutritional interventions, Addiction, 107 (2012) 635-641.

[70] K. Varshneya, C. Carico, A. Ortega, C.G. Patil, The Efficacy of Ketogenic Diet and Associated Hypoglycemia as an Adjuvant Therapy for High-Grade Gliomas: A Review of the Literature, Cureus, 7 (2015) e251.

[71] K. Schwartz, H.T. Chang, M. Nikolai, J. Pernicone, S. Rhee, K. Olson, P.C. Kurniali, N.G. Hord, M. Noel, Treatment of glioma patients with ketogenic diets: report of two cases treated with an IRB-approved energy-restricted ketogenic diet protocol and review of the literature, Cancer Metab, 3 (2015) 3.

[72] E. Lee, H.C. Kang, H.D. Kim, Ketogenic Diet for Children with Epilepsy: A Practical Meal Plan in a Hospital, Clin Nutr Res, 5 (2016) 60-63.

[73] L. Montanaro, D. Trere, M. Derenzini, Nucleolus, ribosomes, and cancer, Am J Pathol, 173 (2008) 301-310.

[74] J.J. Elser, R.W. Sterner, E. Gorokhova, W.F. Fagan, T.A. Markow, J.B. Cotner, J.F. Harrison, S.E. Hobbie, G.M. Odell, L.W. Weider, Biological stoichiometry from genes to ecosystems, Ecol Lett, 3 (2000) 540-550.

[75] Y. Kuang, J.D. Nagy, J.J. Elser, Biological stoichiometry of tumor dynamics: mathematical models and analysis, Discrete & Continuous Dynamical Systems Series B, 4 (2004) 221-240.

[76] D.N. Ward, A.C. Griffin, Phosphorus incorporation into nucleic acids and proteins of liver nuclei of normal and azo dye-fed rats, Cancer Res, 15 (1955) 456-461.

[77] D.A. Guertin, D.M. Sabatini, Defining the role of mTOR in cancer, Cancer Cell, 12 (2007) 9-22.

[78] H. Jin, C.X. Xu, H.T. Lim, S.J. Park, J.Y. Shin, Y.S. Chung, S.C. Park, S.H. Chang, H.J. Youn, K.H. Lee, Y.S. Lee, Y.C. Ha, C.H. Chae, G.R. Beck, Jr., M.H. Cho, High dietary inorganic phosphate increases lung tumorigenesis and alters Akt signaling, Am J Respir Crit Care Med, 179 (2009) 59-68.

[79] C.E. Camalier, M.R. Young, G. Bobe, C.M. Perella, N.H. Colburn, G.R. Beck, Jr., Elevated phosphate activates N-ras and promotes cell transformation and skin tumorigenesis, Cancer Prev Res (Phila), 3 (2010) 359-370.

[80] J. Chudek, A. Nagy, F. Kokot, A. Podwinski, A. Wiecek, E. Ritz, G. Kovacs, Phosphatemia is related to chromosomal aberrations of parathyroid glands in patients with hyperparathyroidism, J Nephrol, 20 (2007) 164-172.

[81] S. Yilmaz, F. Unal, D. Yuzbasioglu, M. Celik, DNA damage in human lymphocytes exposed to four food additives in vitro, Toxicol Ind Health, 30 (2014) 926-937.

[82] N. Schupp, H. Stopper, A. Heidland, DNA Damage in Chronic Kidney Disease: Evaluation of Clinical Biomarkers, Oxid Med Cell Longev, 2016 (2016) 3592042.

[83] A.C. Allison, Lysosomes in cancer cells, J Clin Pathol Suppl (R Coll Pathol), 7 (1974) 43-50.

[84] C. Lopez-Otin, T. Hunter, The regulatory crosstalk between kinases and proteases in cancer, Nat Rev Cancer, 10 (2010) 278-292.

[85] M.C. Mendoza, E.E. Er, J. Blenis, The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation, Trends Biochem Sci, 36 (2011) 320-328.

[86] E.R. Lee, J.Y. Kim, Y.J. Kang, J.Y. Ahn, J.H. Kim, B.W. Kim, H.Y. Choi, M.Y. Jeong, S.G. Cho, Interplay between PI3K/Akt and MAPK signaling pathways in DNA-damaging drug-induced apoptosis, Biochim Biophys Acta, 1763 (2006) 958-968.

[87] H. Yokota, K. Gomi, T. Shintani, Induction of autophagy by phosphate starvation in an Atg11-dependent manner in Saccharomyces cerevisiae, Biochem Biophys Res Commun, 483 (2017) 522-527.

[88] H. Ledford, Cancer theory faces doubts, Nature, 472 (2011) 273.

[89] C.P. Ramirez, D. Fiedler, Investigating the role of inorganic phosphate in tumor metabolism and metastasis, Cancer Metab, 2(Suppl 1) (2014) p53.

[90] S. Iff, J.C. Craig, R. Turner, J.R. Chapman, J.J. Wang, P. Mitchell, G. Wong, Reduced estimated GFR and cancer mortality, Am J Kidney Dis, 63 (2014) 23-30.

[91] C.Y. Jamal, Alteration in bone mineral metabolism in children with acute lymphoblastic leukemia (ALL): A review, BSMMU J, 1 (2008) 29-32.

[92] L.H. Opie, G.D. Lopaschuk, What is good for the circulation also lessens cancer risk, Eur Heart J, 36 (2015) 1157-1162.

[93] M.M. Althaf, The advent of Onco-nephrology - a novel subspecialty, J Renal Inj Prev, 3 (2014) 57-59.

[94] J.W. Eagen, Glomerulopathies of neoplasia, Kidney Int, 11 (1977) 297-303.

[95] R.R. Pascal, P.M. Iannaccone, F.M. Rollwagen, T.A. Harding, S.J. Bennett, Electron microscopy and immunofluorescence of glomerular immune complex deposits in cancer patients, Cancer Res, 36 (1976) 43-47.

[96] M. Weller, R. Stupp, W. Wick, Epilepsy meets cancer: when, why, and what to do about it?, Lancet Oncol, 13 (2012) e375-382.

[97] S.C. Howard, D.P. Jones, C.H. Pui, The tumor lysis syndrome, N Engl J Med, 364 (2011) 1844-1854.

[98] S. Yamada, M. Tokumoto, N. Tatsumoto, M. Taniguchi, H. Noguchi, T. Nakano, K. Masutani, H. Ooboshi, K. Tsuruya, T. Kitazono, Phosphate overload directly induces systemic inflammation and malnutrition as well as vascular calcification in uremia, Am J Physiol Renal Physiol, 306 (2014) F1418-1428.

[99] A. El Maghraoui, F.B. Ebo'o, S. Sadni, A. Majjad, T. Hamza, A. Mounach, Is there a relation between pre-sarcopenia, sarcopenia, cachexia and osteoporosis in patients with ankylosing spondylitis?, BMC Musculoskelet Disord, 17 (2016) 268.

[100] H. Puolijoki, J. Mustonen, E. Pettersson, A. Pasternack, A. Lahdensuo, Proteinuria and haematuria are frequently present in patients with lung cancer, Nephrol Dial Transplant, 4 (1989) 947-950.

[101] P. Pai, J.M. Bone, I. McDicken, G.M. Bell, Solid tumor and glomerulopathy., Q J Med, 89 (1996) 361-367.

[102] P.M. Ronco, Paraneoplastic glomerulopathies: new insights into an old entity, Kidney Int, 56 (1999) 355-377.

[103] J. Mustonen, A. Pasternack, H. Helin, IgA mesangial nephropathy in neoplastic diseases, Contrib Nephrol, 40 (1984) 283-291.

[104] D.M. Burstein, S.M. Korbet, M.M. Schwartz, Membranous glomerulonephritis and malignancy, Am J Kidney Dis, 22 (1993) 5-10.

[105] B. Xie, J. Zhou, G. Shu, D.C. Liu, J. Chen, L. Yuan, Restoration of klotho gene expression induces apoptosis and autophagy in gastric cancer cells: tumor suppressive role of klotho in gastric cancer, Cancer Cell Int, 13 (2013) 18.

[106] B. Xie, J. Chen, B. Liu, J. Zhan, Klotho acts as a tumor suppressor in cancers, Pathol Oncol Res, 19 (2013) 611-617.

[107] M. Kawai, The FGF23/Klotho axis in the regulation of mineral and metabolic homeostasis, Horm Mol Biol Clin Investig, 28 (2016) 55-67.

[108] T. Rubinek, I. Wolf, The Role of Alpha-Klotho as a Universal Tumor Suppressor, Vitam Horm, 101 (2016) 197-214.

[109] M.S. Razzaque, The role of Klotho in energy metabolism, Nat Rev Endocrinol, 8 (2012) 579-587.

[110] R. Goetz, M. Ohnishi, S. Kir, H. Kurosu, L. Wang, J. Pastor, J. Ma, W. Gai, M. Kuro-o, M.S. Razzaque, M. Mohammadi, Conversion of a paracrine fibroblast growth factor into an endocrine fibroblast growth factor, J Biol Chem, 287 (2012) 29134-29146.

[111] R. Goetz, M. Ohnishi, X. Ding, H. Kurosu, L. Wang, J. Akiyoshi, J. Ma, W. Gai, Y. Sidis, N. Pitteloud, O.M. Kuro, M.S. Razzaque, M. Mohammadi, Klotho coreceptors inhibit signaling by paracrine fibroblast growth factor 8 subfamily ligands, Mol Cell Biol, 32 (2012) 1944-1954.

[112] M. Ohnishi, M.S. Razzaque, Dietary and genetic evidence for phosphate toxicity accelerating mammalian aging, FASEB J, 24 (2010) 3562-3571.

[113] T. Nakatani, B. Sarraj, M. Ohnishi, M.J. Densmore, T. Taguchi, R. Goetz, M. Mohammadi, B. Lanske, M.S. Razzaque, In vivo genetic evidence for klotho-dependent, fibroblast growth factor 23 (Fgf23) -mediated regulation of systemic phosphate homeostasis, FASEB J, 23 (2009) 433-441.

[114] T. Nakatani, M. Ohnishi, M.S. Razzaque, Inactivation of klotho function induces hyperphosphatemia even in presence of high serum fibroblast growth factor 23 levels in a genetically engineered hypophosphatemic (Hyp) mouse model, FASEB J, 23 (2009) 3702-3711.

[115] S. Ichikawa, E.A. Imel, M.L. Kreiter, X. Yu, D.S. Mackenzie, A.H. Sorenson, R. Goetz, M. Mohammadi, K.E. White, M.J. Econs, A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis, J Clin Invest, 117 (2007) 2684-2691.

[116] X. Tang, Y. Wang, Z. Fan, G. Ji, M. Wang, J. Lin, S. Huang, S.J. Meltzer, Klotho: a tumor suppressor and modulator of the Wnt/beta-catenin pathway in human hepatocellular carcinoma, Lab Invest, 96 (2016) 197-205.

[117] H. Ligumsky, T. Rubinek, K. Merenbakh-Lamin, A. Yeheskel, R. Sertchook, S. Shahmoon, S. Aviel-Ronen, I. Wolf, Tumor Suppressor Activity of Klotho in Breast Cancer Is Revealed by Structure-Function Analysis, Mol Cancer Res, 13 (2015) 1398-1407.

[118] I. Wolf, S. Levanon-Cohen, S. Bose, H. Ligumsky, B. Sredni, H. Kanety, M. Kuro-o, B. Karlan, B. Kaufman, H.P. Koeffler, T. Rubinek, Klotho: a tumor suppressor and a modulator of the IGF-1 and FGF pathways in human breast cancer, Oncogene, 27 (2008) 7094-7105.

[119] J. Lee, D.J. Jeong, J. Kim, S. Lee, J.H. Park, B. Chang, S.I. Jung, L. Yi, Y. Han, Y. Yang, K.I. Kim, J.S. Lim, I. Yang, S. Jeon, D.H. Bae, C.J. Kim, M.S. Lee, The anti-aging gene KLOTHO is a novel target for epigenetic silencing in human cervical carcinoma, Mol Cancer, 9 (2010) 109.

[120] L. Wang, X. Wang, P. Jie, H. Lu, S. Zhang, X. Lin, E.K. Lam, Y. Cui, J. Yu, H. Jin, Klotho is silenced through promoter hypermethylation in gastric cancer, Am J Cancer Res, 1 (2011) 111-119.

[121] T. Rubinek, M. Shulman, S. Israeli, S. Bose, A. Avraham, A. Zundelevich, E. Evron, E.N. Gal-Yam, B. Kaufman, I. Wolf, Epigenetic silencing of the tumor suppressor klotho in human breast cancer, Breast Cancer Res Treat, 133 (2012) 649-657.

[122] S. Doi, Y. Zou, O. Togao, J.V. Pastor, G.B. John, L. Wang, K. Shiizaki, R. Gotschall, S. Schiavi, N. Yorioka, M. Takahashi, D.A. Boothman, M. Kuro-o, Klotho inhibits transforming growth factor-beta1 (TGF-beta1) signaling and suppresses renal fibrosis and cancer metastasis in mice, J Biol Chem, 286 (2011) 8655-8665.

4) Highlights (mandatory)

Highlights consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters per bullet point including spaces).

Phosphate toxicity can promote tumorigenesis
Phosphate can act as a mitogenic factor to induce tumor cell proliferation
Phosphate can activate growth promoting tumor cell signaling
Phosphate transporters are over-expressed on certain tumor cells
Phosphate regulating factor klotho can act as a tumor suppressor