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# Hereditary disorders of magnesium reveal new proteins implicated in its renal transport

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## INTRODUCTION

Magnesium is the second most common intracellular ion and the fourth most abundant cation in the body. This divalent cation plays an essential role in many metabolic processes such as protein and DNA synthesis and oxidative phosphorylation. It is also a critical cofactor in a high number of enzymatic reactions, and is involved in regulation of ion channels.[1] In normal subjects, an acute change in serum magnesium levels affects parathyroid function: decreased magnesium levels stimulate secretion, while hypermagnesemia inhibits PTH release.[2,3]

Magnesium deficiency therefore affects multiple body functions. Symptoms of magnesium deficiency mainly consist of neuromuscular hyperexcitability ranging from latent to overt tetany and/or seizures,[4] and from simple electrocardiographic changes including prolonged PR and QT intervals to complex cardiac arrhythmia. Magnesium deficiency is a very common problem, found in more than 10% of hospitalized patients, and may occur in up to 65% of patients in intensive therapy units.[5] A complication seen in adult patients with chronic hypomagnesemia is chondrocalcinosis, particularly in the knees, that may lead to joint function impairment.[4]

Magnesium deficiency usually results from magnesium loss, either through the gastrointestinal tract or the kidney. Diseases causing acute or chronic diarrhea, either or not associated to malabsorption, commonly induce magnesium deficiency. Diabetes is probably the most common systemic disease associated to hypomagnesemia. Osmotic diuresis due to glycosuria results in renal loss of magnesium. Different drugs such as diuretics, aminoglycosides,[6] cyclosporin,[7] and cisplatin may also cause renal loss of magnesium.

## RENAL HANDLING OF MAGNESIUM HOMEOSTASIS

Magnesium plasma levels are regulated within a very narrow margin by changes in urinary excretion of this cation in response to intestinal absorption changes. The kidney therefore plays an essential role in magnesium homeostasis.[4,8] Only a small fraction of filtered magnesium is reabsorbed into the proximal tubule (approximately 15% of the filtered load). Most renal reabsorption of magnesium occurs in the thick ascending limb of Henle's loop (± 70%) through a paracellular passive transport (fig. 1) driven by an electric gradient. Approximately 10% of filtered magnesium is reabsorbed into the distal convoluted tubule (DCT) and the connecting tubule by a process of transcellular active transport.[6,8] Apical entry into DCT and connecting tubule cells is mediated by special magnesium-permeable channels called TRPM6 (transient receptor potential cation channel, subfamily M, member 6) that are driven by a favorable transmembrane voltage gradient.[9] The mechanism of basolateral magnesium exit to the interstitium is unknown (fig. 2). Magnesium should be extruded against an unfavorable electrochemical gradient, which is most likely to occur through a $Na^+/Mg^{2+}$ exchanger and/or a $Mg^{2+}$ATPase. Finally, 3%-5% of filtered magnesium is excreted in urine. In hypomagnesemia states, the kidney may reduce magnesium excretion to 0.5% of the filtered load, while in hypermagnesemia it may excrete up to 80% of the filtered load. Despite the significant role play by transepithelial transport mechanisms in magnesium handling, such mechanisms have not been fully elucidated yet.

## HEREDITARY DISORDERS OF MAGNESIUM HANDLING AND NEW PROTEINS IMPLICATED IN MAGNESIUM TRANSPORT

Hereditary primary hypomagnesemia is a rare group of heterogeneous disorders characterized by renal or intestinal magnesium loss with magnesium depletion frequently associated to impaired calcium excretion, resulting in shared symptoms of tetany and generalized seizures. Study of these disorders has allowed for a deeper understanding of the cellular and molecular mechanisms that play a significant role in renal magnesium reabsorption. In recent years, genetic studies on several of these hereditary disorders have revealed four new proteins that are involved in renal magnesium transport: 1) claudin-16, 2) the abovementioned magnesium epithelial channel, TRPM6, 3) the gamma subunit of Na,K-ATPase, and 4) pro-EGF (pro-epidermal growth factor).

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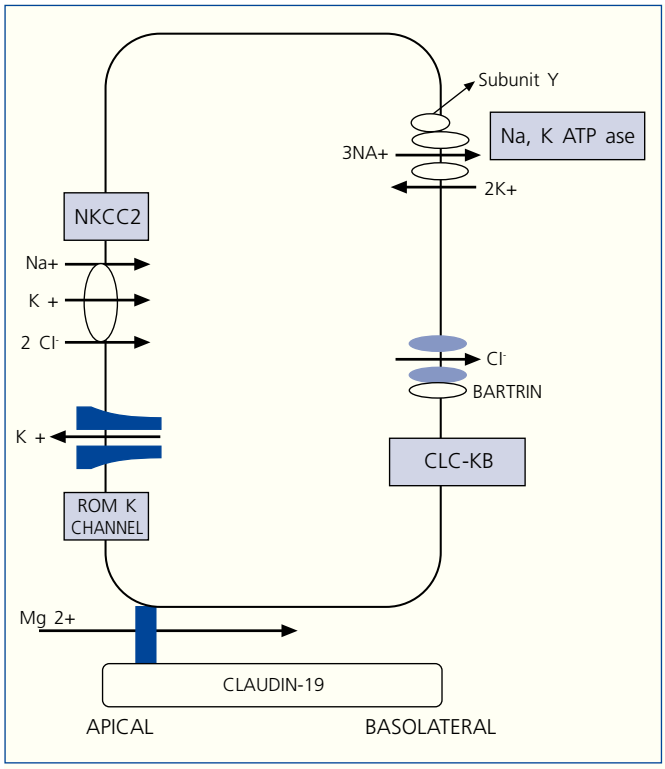


**Figure 1.** Simplified schematic model of transport mechanism of the ascending thick limb of Henle.

## Familial hypomagnesemia with hypercalciuria and nephrocalcinosis and mutations in tight junction proteins claudin-16 and -19

In 1999, a rare syndrome, familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), was found to be caused by mutation of paracellin-1, subsequently called claudin-16.[10] Tubular disorders and progression to renal insufficiency are usually resistant to magnesium replacement and hydrochlorothiazide treatment, but magnesemia may improve with the advance of renal failure.

As previously stated, the bulk of magnesium tubular reabsorption occurs in the ascending thick limb of Henle's loop. This tubular segment consists of a watertight epithelium, which is very important to generate the medullary hyperosmolarity gradient caused by sodium chloride absorption on which subsequent water reabsorption by the collecting tubule depends. Sodium chloride reabsorption depends on the presence in the apical membrane of tubular cells in this region of an electron-neutral cotransporter carrying two chlorines, one potassium, and one sodium (NKCC2), which is the molecular target of the so-called loop diuretics such as furosemide. Potassium must exit again into the tubular lumen through special channels called ROMK (renal outer medullary K channels). This generates and maintains a positive intratubular potential of 6 to 12 mVolt, which in turn drives paracellular reabsorption of divalent cations calcium and magnesium. The finding that the paracellular protein claudin-16, expressed in the tight junctions of the ascending thick limb of Henle's loop, was involved in magnesium reabsorption initially suggested that this protein could be the paracellular route for magnesium reabsorption. When a series of claudin-16 mutations found in FHHNC patients were investigated by expressing them in renal cell lines, most of these mutated proteins were found to be retained within the cell. A few mutant proteins were directed, as normally occurs, towards tight junctions, but these showed a reduced conductivity for magnesium.[11] It was therefore thought that claudin-16 mutations found in FHHNC affected its intracellular traffic or paracellular permeability to magnesium. However, other studies have shown that claudin-16 only has a low permeability to magnesium, but has a high permeability to sodium, and it was postulated that claudin-16 formed a paracellular shunt for sodium in the interstitium to return to the tubular lumen, contributing to the generation of the positive potential in the tubular lumen.[12] This hypothesis was recently evaluated using RNA interference technology to generate a mouse model with a great reduction in claudin-16 expression.[13] This mouse model showed urinary loss of magnesium and calcium, bone mass reduction, and subsequent development of nephrocalcinosis as seen in patients with FHHNC. A detailed analysis of the function of the ascending thick limb of Henle in these mice with no claudin-16 showed a decreased paracellular permeability to sodium with a strong reduction in the lumen-positive potential. These data would show that claudin-16 may be part of the tight junction complex that selectively mediates back diffusion of sodium from interstitium to the lumen of the ascending thick limb of Henle, generating the electropositive luminal potential that is critical for paracellular reabsorption of calcium and magnesium.

In a study on patients with mutations resulting in a complete loss of function of both claudin-16 alleles, they were found to be younger at symptom start as compared to subjects who had an allele providing a partial function.[14] In addition, patients with a complete function loss had a faster impairment of glomerular filtration rate, which caused that more than half of them required renal replacement therapy at 15 years of age, As compared to only 20% of those with residual allele function. Existence of residual claudin-16 function could therefore delay progression to renal failure in patients with FHHNC.

More recently, nine families with severe hypomagnesemia with mutations in the gene encoding claudin-19 have been reported.[15] Claudin-19 is another tight junction protein expressed in renal tubules and eyes.[16] This is why patients with claudin-19 mutations have ocular symptoms such as severe visual impairment, macular coloboma, horizontal nystagmus, and marked myopia which do not occur in patients with claudin-16 mutations. In epithelial cells of pig kidneys, claudin-19 acts as a chloride blocker, while claudin-16 acts as a sodium channel. Claudin-19 mutations found in patients with FHHNC were unable to block permeability to chloride. Coexpression of claudin-16 and -19 generates cation selectivity of the tight junction in a synergistic manner.[17]

## Hypomagnesemia with secondary hypocalcemia and mutations of the magnesium channel TRPM6

This rare autosomal recessive disease (HSH; OMIM 602014), characterized by low serum magnesium levels

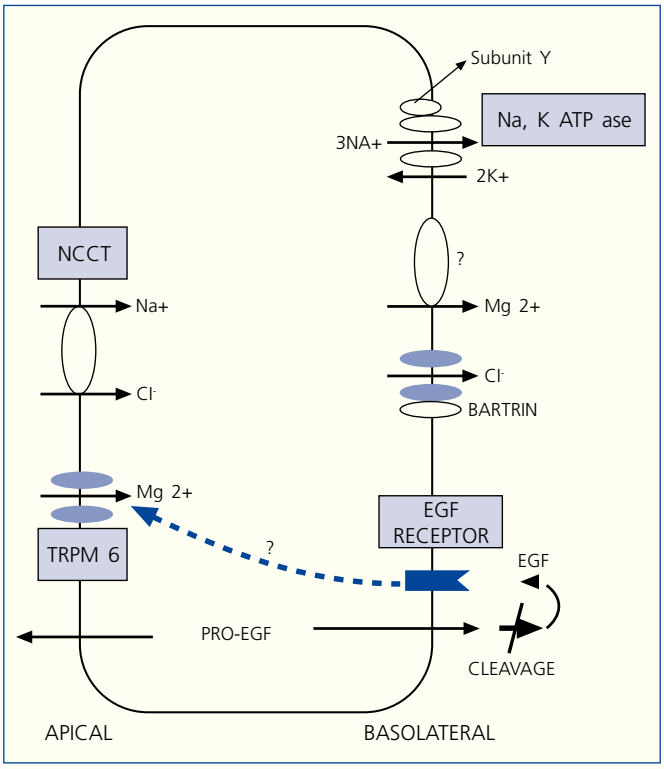


**Figure 2.** Simplified schematic model of transport mechanisms of the distal convoluted tubule.

with a high urinary fractional secretion of magnesium, is caused by nonsense or antisense mutations in the apical magnesium channel, TRPM6.[18] Subsequent studies showed TRPM6 to be a channel permeable to magnesium expressed in the luminal membrane of intestinal epithelium and DCT and connecting tubule.[19] TRPM6 inactivating mutations cause an intestinal absorption impairment combined with renal loss of the cation.

Gitelman syndrome is another hereditary disorder also causing changes in the epithelial magnesium channel. This hereditary disorder is caused by function loss due to mutations in the gene encoding the Na-Cl cotransporter of the distal convoluted tubule (NCCT). It is characterize by hypokalemia, metabolic alkalosis, hypomagnesemia, and hypocalciuria. Hypomagnesemia developing during chronic hydrochlorothiazide treatment and in Na-Cl cotransporter knockout mice, an animal model of Gitelman syndrome, is due to downregulation of the epithelial magnesium channel, TRPM6. Downregulation of this channel may therefore represent a general mechanism involved in the pathogenesis of hypomagnesemia that is associated to inhibition or inactivation of the Na-Cl cotransporter.[20,21]

## Autosomal dominant renal hypomagnesemia with hypocalciuria and mutations in the Na,K-ATPase subunit

In the kidney, Na+, K+-ATPase is an oligomer (alpha/beta/gamma) with equimolar amounts of the alpha and beta essential subunits and a small hydrophobic protein, the gamma subunit. FXYD2 or gamma subunit of Na,K-ATPase belongs to the FXYD family of proteins, which are tissue-specific Na, K-ATPase modulators and include phospholemman (or FXYD1) and CHIF (corticosteroid hormone-induced factor or FXYD4 ). Expression of protein FXYD2 or gamma subunit is essentially restricted to the kidney and has two main variants, gamma a and gamma b. While phospholemman and CHIF increase the apparent affinity of Na, K-ATPase for intracellular Na(+), the gamma subunit decreases sodium affinity.[22] The two variants of the gamma subunit affect the catalytic properties of the pump. Both variants are coexpressed in the proximal tubule and medullary portion of the ascending thick limb of Henle's loop. Distribution of both variants in all other tubular segments differs: only the gamma a variant is present in macula densa and principal cells of the initial parts of the collecting tubule. The gamma b variant is in the cortical portion of the ascending thick limb of Henle's loop.[23] The gamma subunit is an activator of Na+, K+-ATPase in the external medullary zone of the kidney, and its phosphorylation by PKA increases its capacity to stimulate hydrolysis of ATP.[24]

In a large Dutch family with autosomal dominant renal hypomagnesemia associated to hypercalciuria, the disease locus was recently mapped to a 5.6-cM region on chromosome 11q23.[25] After candidate screening, a heterozygous mutation was identified in gene FXYD2, encoding for the gamma subunit of Na(+),K(+)-ATPase, cosegregating with patients from this family, and which was not found in 132 control chromosomes. The mutation leads to a G41R substitution, introducing a charged amino acid residue into the predicted transmembrane region of the gamma subunit protein. Expression studies in insect Sf9 and COS-1 cells showed the mutant gamma subunit to be misrouted and to accumulate in perinuclear structures. In addition to misrouting of mutant G41R, Western blot analysis of Xenopus oocytes expressing either the wild or the mutant type of the gamma subunit showed that a post-translational change was lacking in the mutant gamma subunit. Finally, researchers studied two subjects who lacked a copy of the FXYD2 gene and found that the serum magnesium levels were within the normal range. Retention of mutant gamma subunits in precise intracellular structures was therefore associated to an aberrant post-translational processing. Thus, the G41R mutation in protein FXYD2 causes dominant renal hypomagnesemia associated to hypocalciuria through a negative dominant mechanism. Despite the foregoing, the mechanism by which a mutation in a regulatory protein of the Na(+),K(+)-ATPase pump causes renal magnesium loss has not been elucidated yet.

## Isolated recessive renal hypomagnesemia and mutations in pro-EGF

This disease (IRH) is characterized by low magnesium levels, normocalciuria, and mental retardation with seizures. Groenestetege et al studied two sisters born from asymptomatic inbred parents, which suggested an autosomal recessive pattern.[26] Mutations in other genes previously identified with renal handling of magnesium were ruled out in these patients. Genetic mapping allowed these authors to identify

a critical gap junction with a LOD score of 2.66 at 18.4 cM on chromosome 4 between markers D4S2623 and D4S1575. Among candidate genes located in that region, the EGF (epidermal growth factor) gene was considered highly relevant. EGF sequencing in affected subjects identified a homozygous mutation C3209T in exon 22 that caused substitution of a highly conserved proline by a leucine in the cytoplasmic tail of pro-EGF (P1070L). The EGF gene consists of 24 exons encoding a long precursor protein anchored to the type I membrane that undergoes proteolytic cleavage to be converted into pro-EGF, which eventually generates an acidic 53-amino acid hormone, EGF.[27] EGF belongs to the EGF-like growth factor family, whose members have profound effects upon cell differentiation, and is a potent mitogen.[28] EGF is bound with great affinity to the EGF receptor (EGFR). EGF is very abundant in the DCT and appears to be secreted both to the apical and basolateral sides, while EGFR mainly occurs in the basolateral membrane. Groenestege et al[26] showed that the P1070L mutation in pro-EGF appeared to affect EGF routing and basolateral secretion, whereas apical release was not affected in Madin-Darby canine kidney cells (MDCK). Despite the fact that proline 1,070 may be part of the PXXP motif causing basolateral sorting of pro-EGF, expression of mutated pro-EGF (P1070L) in human embryonic kidney cells (HEK) may also affect EGF formation, suggesting the possibility that the mutation may affect pro-EGF processing.

Regardless of whether the mutation found in patients with IRH causes mistargeting or impairment in pro-EGF processing, Groenestege et al[26] found that EGF markedly increases the activity of the magnesium channel TRPM6. This led the authors to propose a physiological model in which a basal activity of basolateral activation of EGFR is required for TRPM6 activity and apical entry of magnesium. This model is consistent with the hypomagnesemia seen in cancer patients treated with the anti-EGF antibody cetuximab.[29,30] To support this concept, the authors showed that cetuximab also antagonized stimulation of TRPM6 activity by EGF in cultured cells.

## PERSPECTIVE

After many decades of research, in-depth understanding of control of magnesium homeostasis is still lacking. Study of the different hereditary disorders of magnesium has demonstrated new proteins involved in its handling. The most significant finding may perhaps be that EGF acts as an autocrine/paracrine mangesiotropic factor, which opens the way to a better understanding of active magnesium reabsorption in the distal tubule. Pending questions include whether the effect of EGF is exerted through regulation of channel activity or whether it regulates its apical expression, and which are its intracellular signaling pathways. Understanding of all these mechanisms will open the door to a set of therapeutic objectives to be able to manipulate renal magnesium handling.

## REFERENCES

1. Naderi AS, Reilly RF Jr. Hereditary etiologies of hypomagnesemia. *Nat Clin Pract Nephrol* 2008; 4 (2): 80-89.
2. Ferment O, Garnier PE, Touitou Y. Comparison of the feedback effect of magnesium and calcium on parathyroid hormone secretion in man. *J Endocrinol* 1987; 113: 117-122.
3. Cholst IN et al. The influence of hypermagnesemia on serum calcium and parathyroid hormone levels in human subjects. *N Engl J Med* 1984; 310: 1221-1225.
4. Konrad M, Schlingmann KP, Gudermann T. Insights into the molecular nature of magnesium homeostasis. *Am J Physiol Renal Physiol* 2004; 286: F599-F605.
5. Agus ZS. Hypomagnesemia. *J Am Soc Nephrol* 1999; 10: 1616-1622.
6. Zaloga GP, Chernow B, Pock A, Wood B, Zaritsty A, Zucker A. Hypomagnesemia is a common complication of aminoglycoside therapy. *Surg Gynecol Obstet* 1984; 158: 561-565.
7. June CH, Thompson CB, Lkennedy MS, Nims J, Thomas ED. Profound hypomagnesemia and renal magnesium wasting associated with the use of cyclosporine for bone marrow transplantation. *Transplantation* 1985; 39: 620-624.
8. Hoenderop JG, Bindels RJ. Epithelial $Ca^{2+}$ and $Mg^{2+}$ channels in health and disease. *J Am Soc Nephrol* 2005; 16: 15-26.
9. Voets T, Nilius B, Hoef S, Van der Kemp AW, Droogmans G, Bindels RJ, Hoenderop JG. TRPM6 forms the $Mg^{2+}$ influx channel involved in intestinal and renal $Mg^{2+}$ absorption. *J Biol Chem* 2004; 279: 19-25.
10. Simon DB, Lu Y, Choate KA, Velásquez H, Al-Sabban E, Praga M, Casari G, Bettinelli A, Colussi G, Rodríguez Soriano J, McCredie D, Milford D, Sanjad S, Lifton RP. Paracellin-1, a renal tight junction protein required for paracellular Mg2+ resorption. *Science* 1999; 285 (5424): 103-6.
11. Kausalya PJ, Amasheh S, Gunzel D, Wurps H, Muller D, Fromm M, Hunziker W. Disease-associated mutations affect intracellular traffic and paracellular Mg2+ transport function of Claudin-16. *J Clin Invest* 2006; 116: 878-91.
12. Hou J, Paul DL, Goodenough DA. Paracellin-1 and the modulation of ion selectivity of tight junctions. *J Cell Sci* 2005; 118: 5109-18.
13. Hou J, Shan Q, WangT, Gomes AS, Yan Q, Paul DL, Bleich M, Goodenough DA. Transgenic RNAi depletion of claudin 16 and the renal handling of magnesium. *J Biol Chem* 2007; 282: 17114-22.
14. Konrad M, Hou J, Weber S, Dotsch J, Kari JA, Seemann T et al. *CLDN16* Genotype predicts renal decline in familial hypomagnesemia with hypercalciuria and nephrocalcinosis. *J Am Soc Nephrol* 2008; 19 (1): 171-81.
15. Konrad M, Schaller A, Seelow D, Pandey AV, Waldegger S, Lesslauer A et al. Mutations in the tight-junction gene claudin 19 (CLDN19) area associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am J Hum Genet* 2006; 79: 949-957.
16. Angelow S. Renal localization and function of the tight junction protein claudin-19. *Am J Physiol Renal Physiol* 2007; 293: F166-F177.
17. Hou J, Renigunta A, Konrad M, Gomes AS, Scheeberger EE, Paul DL, Waldegger S, Goodenough DA. Claudin-16 and claudin-19 interact and form a cation-selective tight junction complex. *J Clin Invest* 2008; 118 (2): 619-28.
18. Schlingmann KP et al. Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat Genet* 2002; 31: 166-170.
19. Walder RY et al. Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. *Nat Genet* 2002; 31: 171-174.
20. Simon DB et al. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the $K^+$ channel, ROMK. *Nat Genet* 1996; 14: 152-156.
21. Nijenhuis T, Vallon V, Van der Kemp A, Loffing J, Hoenderop J GJ, Bindels RJM. Enhanced passive $Ca^{2+}$ reabsorption and reduced $Mg^{2+}$ channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. *J Clin Invest* 2005; 115 (6): 1651-8.
22. Geerinng K. Function of FXYD proteins, regulators of Na, K-ATPase. *J Bioenerg Biomembr* 2005; 37 (6): 387-92.
23. Farman N, Fay M, Cluseaud F. Cell-specific expression of three members of the FXYD family along the renal tubule. *Ann N Y Acad Sci* 2003; 986: 4428-436.
24. Cortés VF, Veiga-Lopes FE , Barrabin H, Alves-Ferreira M, Fontes CF. The gamma subunit of Na+, K+-ATPase: role on ATPase activity and regulatory phosphorylation by PKA. *Int J Biochem Cell Biol* 2006; 38 (11): 1901-13.
25. Meij IC, Koenderink JB, De Jong JC, De Pont JJ, Monnens LA, Van Den Heuvel LP, Knoers NV. Dominant isolated renal magnesium loss is caused by misrouting of the Na+,K+-ATPase gamma-subunit. *Ann N Y Acad Sci* 2003; 986: 437-43.

26. Groenestege WM, Thébault S, Van der Wijst J, Van den Berg D, Janssen R, Teipar S, Van den Heuvel LP, Van Cutsem E, Hoenderop JG, Knoerss NV, Bindels RJ. Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest* 2007; 117 (8): 2260-7.
27. Bell GL. Human epidermal growth factor precursor: cDNA sequence, expression *in vitro* and gene organization. *Nucleic Acids Res* 1986; 14: 8427-8446.
28. Gray A, Dull TJ, Ullrich A. Nucleotide sequence of epidermal growth factor cDNA predicts a 128,000-molecular weight protein precursor. *Nature* 1983; 3033: 722-725.
29. Schrag D, Chunng KY, Flombaum C, Saltz L. Cetuximab therapy and symptomatic hypomagnesemia. *J Natl Cancer Ins* 2005; 97: 1221-1224.
30. Tejpar S, Piessevaux H, Claes K, Piront P, Hoenderop JG, Verslype C, van Cutsem E. Magnesium wasting associated with epithermal-growth —factor receptor-targeting antibodies in colorectal cancer: a prospective study. *Lancet Oncol* 2007; 8: 387-394.