

## QUANTITATIVE ANALYSIS OF *SLC34A2* EXPRESSION IN DIFFERENT TYPES OF OVARIAN TUMORS

M. Shyian<sup>1,2,\*</sup>, V. Gryshkova<sup>2</sup>, O. Kostianets<sup>1,2</sup>, V. Gorshkov<sup>3</sup>, Yu. Gogolev<sup>3</sup>, I. Goncharuk<sup>4</sup>,  
S. Nespryadko<sup>4</sup>, L. Vorobjova<sup>4</sup>, V. Filonenko<sup>2</sup>, R. Kiyamova<sup>2</sup>

<sup>1</sup>Taras Shevchenko National University of Kyiv, Kyiv, 01601, Ukraine

<sup>2</sup>Department of Cell Signaling, Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, 03680, Ukraine

<sup>3</sup>Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia

<sup>4</sup>National Cancer Institute, Ministry of Health of Ukraine, Kyiv, 03022, Ukraine

**Aim:** The main purpose of this study was to estimate the *SLC34A2* gene expression in normal ovary and different types of ovarian tumors. **Methods:** We have investigated *SLC34A2* gene expression level in papillary serous, endometrioid, unspecified adenocarcinomas, benign tumors, and normal ovarian tissues using real-time PCR analysis. Differences in gene expression were calculated as fold changes in gene expression in ovarian carcinomas and benign tumors compared to normal ovary. **Results:** We have found that *SLC34A2* gene was highly expressed in well-differentiated endometrioid and papillary serous ovarian carcinomas compared to low-differentiated endometrioid carcinomas, benign serous cystadenomas and normal ovary. Analysis of *SLC34A2* gene expression according to tumor differentiation level (poor- and well-differentiated) showed that *SLC34A2* is up-regulated in well-differentiated tumors. **Conclusion:** Upregulation of *SLC34A2* gene expression in well-differentiated tumors may reflect cell differentiation processes during ovarian cancerogenesis and could serve as potential marker for ovarian cancer diagnosis and prognosis.

**Key Words:** NaPi2b, *SLC34A2*, gene expression, ovarian cancer, normal ovary.

The human sodium-dependent phosphate transport protein 2b — NaPi2b, (NPT11b, NAPI-3B, FLJ90534, NAPI-11b) or solute carrier family 34 member 2 is a transmembrane protein with at least eight highly hydrophobic helical regions that form channel structure via homodimeric assembly [1, 2]. Na/Pi-cotransport via NaPi2b transporter is electrogenic with a likely stoichiometry of 3Na:1Pi [3].

NaPi2b is coded by the *SLC34A2* gene and plays an important role in the maintenance of the overall phosphate homeostasis that is essential for proper cellular functions such as DNA synthesis, cell signaling, bone formation etc [3, 4]. NaPi2b is highly abundant in the brush-border membrane (BBM) of small intestine where it is involved in transcellular flux of inorganic phosphates via apical membrane of epithelial cells [3, 5].

Besides the small intestine, expression of NaPi2b has been described predominantly on mRNA level in some organs of mammals including lung, pancreas, kidney, ovary, placenta, uterus, testis, secreting mammary gland, thyroid gland, salivary gland, bone, and epididymus [4–13] where its physiological role remains to be defined.

NaPi2b expression profile in normal tissues could be supplemented with sites of expression of MX35 antigen because a molecular identity of both proteins recently was proved by several techniques [14]. MX35 antigen expression was described by immuno-

histochemical analysis with correspondent MX35 mAbs in several tissues of epithelial origin. These sites, along with the already described for NaPi2b include cervix, Fallopian tubes and sweat glands [15]. Impairments of *SLC34A2* regulation and mutations in this gene might lead to development of different pathologies. For instance, several mutations of *SLC34A2* gene were reported to be associated with testicular and pulmonary alveolar microlithiasis [16–18] and downregulation of the NaPi2b cotransporter was associated with hypophosphatemia [19, 20].

There are plenty of data about aberrant *SLC34A2* expression in cancer cells. The downregulation of NaPi2b transporter was revealed in non-small cell lung carcinomas by RT-PCR analysis [21]. Recently, a strong overexpression of *SLC34A2* in breast cancer and papillary thyroid cancer was found using real-time RT-PCR [22–24]. Rangel *et al.* using SAGE and Northern-blot analysis reported that *SLC34A2* expression was clearly restricted to the ovarian tumors and certain ovarian cell lines but absent in normal ovarian tissues [25]. Using real-time RT-PCR analysis authors showed that NaPi2b expression in serous ovarian carcinomas was statistically associated with tumor grade and more differentiated tumors tended to express higher levels of *SLC34A2* mRNA [25]. Papillary serous carcinomas are the most common ovarian tumors and represent about 50–60% of all epithelial ovarian cancers (EOC), and the remaining epithelial ovarian carcinomas exhibit endometrioid (25%), mucinous (4%) and clear cell (4%) histology [26]. It was considered that EOC may arise from ovarian surface epithelium (OSE) that has “uncommitted” phenotype and retains the capacity to differentiate into different types of cells in response to environmental signals. During ovarian cancerogenesis, the epithelium of ovary could differentiate into

Received: May 17, 2011.

\*Correspondence: E-mail: m.a.shyian@gmail.com

**Abbreviations used:** ACTB – actin; AGPC – acid guanidinium thiocyanate-phenol-chlorophorm; Ct – cycle threshold; EOC – epithelial ovarian cancer; mAbs – monoclonal antibodies; NaPi2b (MX35, *SLC34A2*) – sodium-dependent phosphate transport protein 2b; OSE – ovarian surface epithelium; SAGE – serial analysis of gene expression.

fallopian tube epithelium (papillary serous tumors), endometrial epithelium (endometrioid tumors), colonic or endocervical epithelium (mucinous tumors) and component of endometriosis (clear cell tumors) [27].

Recently we have described NaPi2b expression at mRNA and protein levels in different types of ovarian carcinomas (serous, endometrioid and mucinous) using immunohistochemistry, Western-blot and conventional PCR analysis. We showed that NaPi2b protein expression detected by anti-NaPi2b mAbs [28] increased in well-differentiated serous and endometrioid tumors compared to poor-differentiated tumors at protein level but almost uniformly expressed in these ovarian tumors at mRNA level [29].

To estimate a quantitative mRNA expression of NaPi2b cotransporter in different histological types of ovarian tumors and normal ovary we have performed investigation of the *SLC34A2* gene expression using quantitative real-time RT-PCR analysis.

## MATERIALS AND METHODS

**Tissue samples.** Ovarian tissue samples, in particular, ovarian carcinomas (n = 14), benign serous cystoadenomas (n = 4) and normal ovarian tissues (n = 3) were obtained during surgical treatment of correspondent patients at the National Cancer Institute (Kyiv, Ukraine). The mean age of patients with ovarian tumors was 47 years (range 22–69 years). Normal ovarian tissue samples (n = 3), obtained during surgical treatment of the patients with endometrial cancer (mean age 46 years, range 19–69 years) were used as the control. Tissue specimens were frozen immediately in liquid nitrogen and stored at –80 °C until further analysis. The histological types of epithelial ovarian cancer and cell differentiation status were confirmed by histopathological examination by clinical pathomorphologists at the Department of Pathology, National Cancer Institute (Kyiv, Ukraine) according Christofer Fletcher's Third Edition of Diagnostic Histopathology of Tumors, 2007. The study protocol was approved by the Ethics Committees of the Institute of Molecular Biology and Genetics and National Cancer Institute.

**Isolation of RNA.** RNA was purified by a standard procedure using AGPC (acid guanidinium thiocyanate-phenol-chlorophorm) method [30]. Tissue samples (100–150 mg) were homogenized in liquid nitrogen and mixed with 1 ml of denaturation solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 5% sarcosyl, 0.1 M 2-mercaptoethanol). Sequentially, 0.1 ml of 2 M sodium acetate, pH 4, 1 ml of phenol (water saturated), and 0.2 ml of chloroform-isoamyl alcohol mixture (49:1) were added to the homogenate, with thorough mixing after the addition of each reagent. The final suspension was shaken for 10 s and cooled on ice for 15 min. Samples were centrifuged at 10,000g for 20 min at 4 °C. The aqueous phase with RNA was transferred to a fresh tube and RNA was precipitated with 1 ml of isopropanol at –20 °C for 1 h. After sedimentation at 10,000g the resulting RNA pellet was dissolved in 0.3 ml of the same denaturation solu-

tion and precipitated with 1 volume of isopropanol at –20 °C for 1 h. After centrifugation for 10 min at 4 °C the RNA pellet was washed with 75% ethanol, sedimented, air dried, and dissolved in 50 µl nuclease-free water.

RNA purity and quality was confirmed by measuring spectrophotometrically A260/A280 ratios, and by 1% agarose-formaldehyde denaturing gel electrophoresis. Total RNA yield was quantitated spectrophotometrically.

**Preparation of cDNA from RNA samples.** Total RNA (3 µg) was converted to cDNA with M-MuLV Reverse Transcriptase (Fermentas) at 37 °C for 60 min using oligo(dT)18 primers in 20 µl reaction volume according to the standard protocol of manufacturer.

**Real-time RT-PCR analysis.** Gene-specific TaqMan probes and PCR primers were designed using NCBI software Primer-BLAST (URL: <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) (Table).

**Table.** Gene-specific TaqMan probes and PCR primers

Gene	Oligonucleotide	Oligonucleotide sequence (5' – 3')	Length
<i>SLC34A2</i>	Forward primer	ttg-gag-gaa-aaa-tgg-cag-gac-ag	23
	Reverse primer	gca-aga-gca-cca-aca-cgg-aca-g	22
	Probe	(FAM)cc-cga-aca-g(T-BHQ1)g-agc-aat-gaa-gag-gac-acc	29
<i>ACTB</i>	Forward primer	ggc-acc-cag-cac-aat-gaa-g	19
	Reverse primer	gcc-gat-cca-cac-gga-gta-ct	20
	Probe	(FAM)tc-aag-atc-a(T-BHQ1)t-gct-cct-cct-gag-cgc	26

The real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) procedure was carried out in 25 µl of reaction mix containing diluted 1:10 cDNA mixture (3 µg — 2 µl), forward and reverse primers (20 pmole each), gene-specific TaqMan probe (20 pmole), 10 x Taq Buffer (2.5 µl), 10 mM dNTP mix (5 nmole — 0.5 µl), Taq DNA Polymerase 5 u/µl (1.25 u — 0.25 µl), 25 mM MgCl<sub>2</sub> (50 nmole — 2 µl) and nuclease-free water. The following thermal conditions were applied: 95 °C for initial denaturation (30 s) and 40 cycles consisting of 95 °C denaturation (10s), 55 °C annealing (5 s) and 60 °C extension (60s). Thermal cycling and fluorescent monitoring were performed using iCycler iQ5 (Bio-Rad, CA, USA). Amplified products were separated on a 1% agarose gel and visualized.

**Calculations.** The point at which the PCR product is first detected above a fixed threshold, termed cycle threshold (Ct), was determined for each sample. When PCR product was not detected above a fixed threshold we defined its Ct as 40 (number of final PCR cycle).

To determine the quantity of gene-specific transcripts present in ovarian tumor cDNA relative to normal ovarian tissue, their respective Ct values were first normalized by subtracting the Ct value obtained from the actin (*ACTB*) endogenous control ( $\Delta Ct = Ct \text{ SLC34A2} - Ct \text{ ACTB}$ ). For 3 normal ovarian cDNA samples normalized  $\Delta Ct$  was calculated as the mean value. The relative concentrations of gene-specific mRNAs in ovarian cancer cDNAs compared to normal ovarian tissue ( $\Delta\Delta Ct$ ) were calculated by subtracting the normalized mean  $\Delta Ct$  value obtained for normal ovarian cDNAs from those obtained for each of 17 ovarian tumor samples ( $\Delta\Delta Ct = \Delta Ct \text{ of tumor} - \text{mean } \Delta Ct$

for 3 normal ovaries), and the relative concentration was determined as  $2^{-\Delta\Delta Ct}$  [31].

**Immunohistochemical analysis.** Representative sections of tumor samples were prepared from paraffin blocks and stained with hematoxylin-eosin according as previously described [29]. Endogenous peroxidase was quenched with  $H_2O_2$  (3%) in 0.01% PBS. After blocking of nonspecific binding by avidin–biotin blocking solution (Vector Laboratories, Burlingame, CA, USA), tissue sections were incubated overnight at 4 °C with anti-NaPi2b mAb (10  $\mu$ g/ml). Then, sections were incubated with biotinylated secondary antibodies for 2 h at room temperature (1 : 400, goat anti-mouse biotinylated IgG, Sigma), followed by incubation with avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA). The immune complexes were developed with diaminobenzidine solution. Hematoxylin was used for counterstaining. Prepared slides were examined with the use of Zeiss Universal microscope (Zeiss, Germany); images were captured using digital AxioCam software.

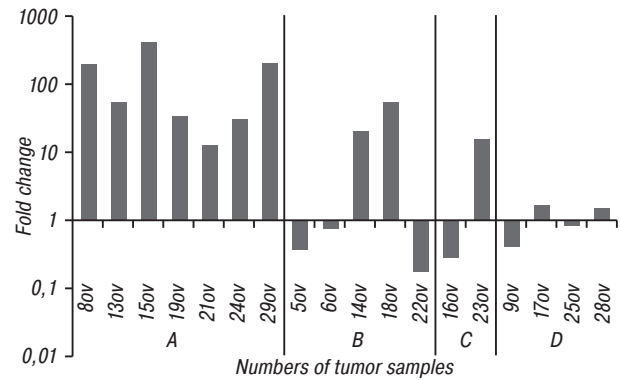
## RESULTS

The expression of the *SLC34A2* gene in ovarian carcinomas ( $n = 14$ ), benign serous cystadenomas ( $n = 4$ ) and normal tissues ( $n = 3$ ) was examined at mRNA level by real-time RT-PCR. In a panel of 14 ovarian carcinomas, there were 7 well-differentiated papillary serous tumors, 5 endometrioid tumors (2 well-differentiated and 3 poor-differentiated carcinomas), and 2 unspecified poor-differentiated adenocarcinomas. Differences in *SLC34A2* gene expression were calculated as fold changes in gene expression in ovarian carcinomas and benign serous cystadenomas compared to three normal ovaries (27 ov, 30 ov, and 35 ov). Since the PCR product in these three samples of normal ovary was not detected above a fixed threshold, which means that NaPi2b is not expressed in normal ovary or is expressed at very low levels we have defined its Ct as 40 in our calculations (see Materials and Methods).

During this study we observed overexpression of the *SLC34A2* gene in all papillary serous tumors compared to normal ovarian tissues (30.5–398.1 with mean 152.9-fold increase) (Fig. 1, A). In addition, we detected a heterogeneous level of the *SLC34A2* gene expression in endometrioid tumors, unspecified poor-differentiated adenocarcinomas and benign tumors compared to normal ovarian tissues (Fig. 1, B, C, and D). It should be noted that the level of *SLC34A2* gene expression in benign ovarian tumors differed slightly compared to normal ovary, in contrast to the expression level of *SLC34A2* gene in ovarian carcinomas where it differed significantly compared to normal tissues.

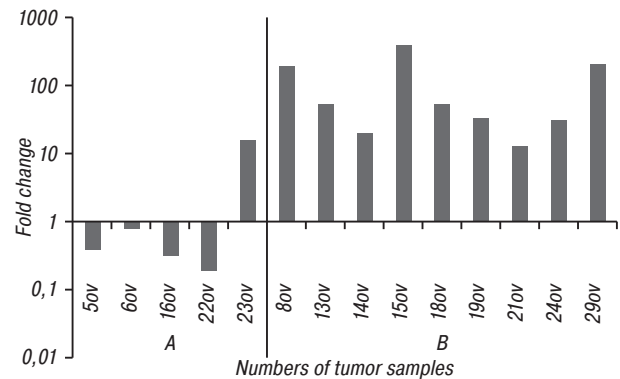
Figure 2 demonstrates *SLC34A2* gene expression in the ovarian carcinoma samples grouped according to their differentiation level. *SLC34A2* gene was highly expressed in all well-differentiated papillary serous tumors and well-differentiated endometrioid tumor samples 14ov and 18ov (19.8–54.1 with mean 36.9-fold increase) compared to poor-differentiated endometri-

oid tumor samples 5ov, 6ov and 22ov (0.18–0.75 with mean 0.44-fold decrease) and poor-differentiated adenocarcinoma tumor sample 16ov (0.29) (Fig. 2).



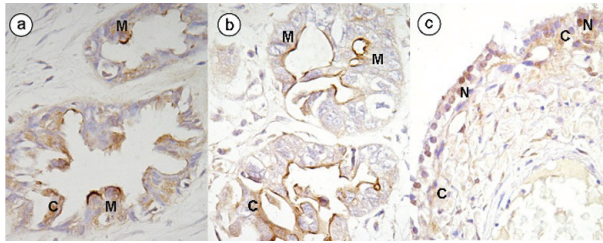
**Fig. 1.** Real-time RT-PCR results for relative expression level of *SLC34A2* in ovarian tumor cDNA samples grouped according to histomorphological type. The mean of *SLC34A2* expression level in 3 normal tissues referred as 1 in all cases. A. Papillary serous carcinomas (8ov, 13ov, 15ov, 19ov, 21ov, 24ov, 29ov); B. Endometrioid carcinomas (14ov, 18ov — well-differentiated; 5ov, 6ov, 22ov — poor-differentiated); C. Unspecified adenocarcinomas (16ov, 23ov — poor-differentiated); D. Benign tumors (9ov, 17ov; 25ov, 28ov — serous cystadenomas)

Data concerning mRNA expression of *SLC34A2* in serous and endometrioid ovarian tumors accurately correlated with NaPi2b protein expression in these types of ovarian tumors detected by Western-blot and immunohistochemical analyses in our previous investigation [29]. Summarizing the results we can conclude that *SLC34A2* gene is up-regulated in well-differentiated ovarian carcinomas compared with poor-differentiated ovarian carcinomas and benign serous cystadenomas both at the mRNA and protein levels.



**Fig. 2.** Real-time RT-PCR results for relative expression level of *SLC34A2* in ovarian cancer samples grouped according to tumor differentiation level. The mean of *SLC34A2* expression level in 3 normal tissues referred as 1 in all cases. A. Poor-differentiated ovarian tumors; B. Well-differentiated ovarian tumors

Immunohistochemical analysis with anti-NaPi2b monoclonal antibodies in our previous investigation and in this study has shown that NaPi2b is expressed predominantly at the surface membrane (M) of cancer cells in well-differentiated serous and endometrioid ovarian carcinomas (Figures 3 a, b) [29]. In addition to membrane localization, in some cases we observed a weak NaPi2b positive staining in the cytoplasm (C) and the nuclei (N) of cancer cells and cells of certain benign tumors (Figures 3 a, b, c).



**Fig. 3.** Immunohistochemical analysis of NaPi2b expression in ovarian tumors: a, serous papillary carcinoma; b, well-differentiated endometrioid carcinoma; c, serous cystadenoma. NaPi2b positive staining at the membrane of cells marked as M, in cytoplasm and in nuclei as C and N correspondently. Magnification x 400

## DISCUSSION

In our previous study, we have analyzed NaPi2b expression at protein and mRNA levels in different types of epithelial ovarian cancer and normal ovarian tissues using Western blot, immunohistochemical and qualitative RT-PCR techniques [29]. We have shown a differential expression profile of NaPi2b phosphate transporter at protein level in various histological types of epithelial ovarian cancer and have not identified any protein expression in normal ovary. Interestingly, we didn't reveal any correlation between NaPi2b protein expression and *SLC34A2* mRNA expression in ovarian tumor samples detected by conventional RT-PCR analysis [29]. This contradiction could be explained in part by putative regulation of NaPi2b expression at the posttranscriptional level [32] in ovarian tumors or by limitation of qualitative RT-PCR analysis. The main purpose of this investigation was to perform quantitative analysis of the *SLC34A2* gene expression by real-time polymerase chain reaction in samples of normal ovary and ovarian tumors.

In this study we have analysed the same tissue samples that were used in our previous investigation [29] and have shown a heterogeneous level of *SLC34A2* gene expression in ovarian tumor samples of different histomorphological types using quantitative real-time RT-PCR analysis in contrast to previous studies where conventional RT-PCR analysis which showed uniform expression *SLC34A2* in these ovarian tumors has been used. mRNA expression of *SLC34A2* in serous and endometrioid ovarian carcinomas, benign serous cystoadenomas as well as in normal ovary accurately correlated with protein expression level that was detected in our previous investigation of these tumor samples by Western-blot and immunohistochemical analyses. These data clearly indicate that the use of quantitative analysis is more sensitive for studying of *SLC34A2* gene expression profile in different types of ovarian tumor samples and in normal ovary. In addition, analysis of *SLC34A2* gene expression according to tumor differentiation level (poor- and well-differentiated) in the endometrioid and serous histological types of ovarian cancer as well as in unspecified ovarian adenocarcinomas showed that *SLC34A2* is up-regulated in well-differentiated tumors. Thus, our results are well consistent with

our previous data about NaPi2b protein expression profile in different types of ovarian carcinomas and data reported by Rangel *et al.* [25] concerning the various *SLC23A3* gene expression profile in serous ovarian carcinomas according to differentiation level. In addition to data Rangel *et al.* [25], we have showed heterogeneous *SLC34A2* expression profile according to differential level in endometrioid tumors as well.

According to immunohistochemical analysis phosphate transporter protein was located predominantly at the surface membrane of cancer cells, but in a few cases cytoplasmic and nuclear localization of NaPi2b in ovarian cancer cells was also observed. Since NaPi2b function in cancer cells has not been studied so far we can only suggest that NaPi2b might be involved in transport of inorganic phosphate from the peritoneal cavity fluid via surface membrane of cancer cells, while NaPi2b function in the cytoplasm and nuclei of cancer cells is completely unclear. NaPi2b function in cancer cells should be investigated in further studies.

Thus, quantitative real-time PCR and immunohistochemical analyses allowed us to investigate more detailed features of NaPi2b expression in different types of ovarian cancer. We have shown that *SLC34A2* gene expression detected by real-time RT-PCR analysis is up-regulated in well-differentiated ovarian carcinomas that correlates with NaPi2b protein expression in cancer cells of the same tumor samples. Taking into account that better differentiated tumors usually have better prognosis, evaluation of *SLC34A2* gene expression could serve as a potential marker for ovarian cancer diagnosis and prognosis.

## REFERENCES

1. Xu H, Collins JF, Bai L, *et al.* Regulation of the human sodium-phosphate cotransporter NaPi2b gene promoter by epidermal growth factor. *Am J Physiol* 2001; **280**: 628–36.
2. Hilfiker H, Hattenhauer O, Traebert M, *et al.* Characterization of a new murine type II sodium phosphate cotransporter expressed in mammalian small intestine. *Proc Natl Acad Sci USA* 1998; **95**: 14564–9.
3. Murer H, Forster I, Biber J. The sodium phosphate cotransporter family SLC34. *Pflügers Arch — Eur J Physiol* 2004; **447**: 763–7.
4. Gupta A, Tenenhouse H, Hoag H, *et al.* Identification of the type II Na<sup>+</sup>-Pi cotransporter (Npt2) in the osteoclast and the skeletal phenotype of Npt2<sup>-/-</sup> mice. *Bone* 2001; **29**: 467–76.
5. Giral H, Caldas Y, Sutherland E, *et al.* Regulation of rat intestinal Na-dependent phosphate transporters by dietary phosphate. *Am J Physiol Renal Physiol* 2009; **297**: 1466–75.
6. Feild JA, Zhang L, Brun KA, *et al.* Cloning and functional characterization of a sodium-dependent phosphate transporter expressed in human lung and small intestine. *Biochem Biophys Res Commun* 1999; **258**: 578–82.
7. Homann V, Rosin-Steiner S, Stratmann T, *et al.* Sodium-phosphate cotransporter in human salivary glands: molecular evidence for the involvement of NPT2b in acinar phosphate secretion and ductal phosphate reabsorption. *Arch Oral Biol* 2005; **50**: 759–68.
8. Ikegami M, Falcone A, Whitsett JA, *et al.* STAT-3 regulates surfactant phospholipid homeostasis in normal lung and during endotoxin-mediated lung injury. *J Appl Physiol* 2008; **104**: 1753–60.

9. **Traebert M, Hattenhauer O, Murer H, et al.** Expression of a type II sodium-phosphate cotransporter in murine type II alveolar epithelial cells. *Am J Physiol* 1999; **277**: 868–73.
10. **Huber K, Muscher A, Breves G.** Sodium-dependent phosphate transport across the apical membrane of alveolar epithelium in caprine mammary gland. *Comp Biochem Phys* 2007; **146**: 215–22.
11. **Frei P, Gao B, Hagenbuch B, et al.** Identification and localization of sodium-phosphate cotransporters in hepatocytes and cholangiocytes of rat liver. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: 771–8.
12. **Lundquist P, Murer P, Biber J, et al.** Type II Na<sup>+</sup>-Pi cotransporters in osteoblast mineral formation: regulation by inorganic phosphate. *Cell Physiol Biochem* 2007; **19**: 43–56.
13. **Xu Y, Yeung CH, Setiawan I, et al.** Sodium-inorganic phosphate cotransporter NaPi2b in the epididymis and its potential role in male fertility studied in a transgenic mouse model. *Biol Reprod* 2003; **69**: 1135–41.
14. **Yin BWT, Kiyamova R, Chua R, et al.** Monoclonal antibody MX35 detects the membrane transporter NaPi2b (SLC34A2) in human carcinomas; a new target for cancer immunotherapy. *Cancer Immun [serial online]* 2008; 8:3. URL: <http://www.cancerimmunity.org/v8p3/080103.htm>.
15. **Mattes MJ, Look K, Furukawa K, et al.** Mouse monoclonal antibodies to human epithelial differentiation antigens expressed on the surface of ovarian carcinoma ascites cells. *Cancer Res* 1987; **47**: 6741–50.
16. **Corut A, Senyigit A, Ugur SA, et al.** Mutations in *SLC34A2* cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis. *Am J Hum Genet* 2006; **79**: 650–6.
17. **Huqun, Izumi S, Miyazawa H, et al.** Mutations in the *SLC34A2* gene are associated with pulmonary alveolar microlithiasis. *Am J Respir Crit Care Med* 2007; **175**: 263–8.
18. **Yang Y, Qiao JH, An JH, et al.** Detection of *SLC34A2* in patients with pulmonary alveolar microlithiasis and the effect of *SLC34A2* on transportation of calcium and phosphate in human alveolar epithelial cells. *Zhonghua Jie He He Hu Xi Za Zhi* 2008; **31**: 908–11.
19. **Konno Y, Moore R, Kamiya N, et al.** Nuclear xenobiotic receptor PXR-null mouse exhibits hypophosphatemia and represses the Na/Pi-cotransporter *SLC34A2*. *Pharmacogenet Genomics* 2010; **20**: 9–17.
20. **Onishi T, Okawa R, Ogawa T, et al.** Phex mutation causes the reduction of npt2b mRNA in teeth. *J Dent Res* 2007; **86**: 158–62.
21. **Kopantzev EP, Monastyrskaya GS, Vinogradova TV, et al.** Differences in gene expression levels between early and later stages of human lung development are opposite to those between normal lung tissue and non-small lung cell carcinoma. *Lung Cancer* 2008; **62**: 23–34.
22. **Chen DR, Chien SY, Kuo SJ, et al.** *SLC34A2* as a novel marker for diagnosis and targeted therapy of breast cancer. *Anticancer Res* 2010; **30**: 4135–40.
23. **Galeza-Kulik M, Zebracka J, Szpak-Ulczo S, et al.** Expression of selected genes involved in transport of ions in papillary thyroid carcinoma. *Endokrynol Pol* 2006; **57**: 26–31.
24. **Kim HS, Kim do H, Kim JY, et al.** Microarray analysis of papillary thyroid cancers in Korean. *Korean J Intern Med* 2010; **25**: 399–407.
25. **Rangel LB, Sherman-Baust CA, Wernyj RP, et al.** Characterization of novel human ovarian cancer-specific transcripts (HOSTs) identified by serial analysis of gene expression. *Oncogene* 2003; **22**: 7225–32.
26. **Farley J, Ozburn LL, Birrer MJ.** Genomic analysis of epithelial ovarian cancer. *Cell Res* 2008; **18**: 538–48.
27. **Auersperg N, Wong AS, Choi KC, et al.** Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocr Rev* 2001; **22**: 255–88.
28. **Kiyamova R, Gryshkova V, Ovcharenko G, et al.** Development of monoclonal antibodies specific for the human sodium-dependent phosphate cotransporter NaPi2b. *Hybridoma* 2008; **27**: 277–84.
29. **Gryshkova V, Goncharuk I, Gurtovyy V, et al.** The study of phosphate transporter NaPi2b expression in different histological types of epithelial ovarian cancer. *Exp Oncol* 2009; **31**: 1–6.
30. **Chomczynski P, Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156–9.
31. **Kubista M, Andrade JM, Bengtsson M, et al.** The real-time polymerase chain reaction. *Mol Aspects Med* 2006; **27**: 95–125.
32. **Xu H, Bai L, Collins JF, et al.** Age-dependent regulation of rat intestinal type IIb sodium-phosphate cotransporter by 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub>. *Am J Physiol Cell Physiol* 2002; **282**: 487–93.