© IJHG 2020 Int J Hum Genet, 20(3): 132-137 (2020)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2020/20.03.752

Arf6 Expression in the Tissues of Patients with Colorectal Cancer

Turkan Gurer[1*], Alper Aytekin[2] and Yasmin Alahdab[1]

[1]Department of Biology, Faculty of Art and Science, Gaziantep University, 27310, Gaziantep, Turkey
[2]Department of General Surgery, School of Medicine, Gaziantep University, 27310, Gaziantep, Turkey

KEYWORDS Clinicopathological Features. Colorectal Tumor. Gene Expression. Real-Time PCR. Small GTPase

ABSTRACT Colorectal cancer is one of the most common types of cancer in the world and the molecular mechanism of colorectal cancer has not been clearly elucidated yet. Adenosine diphosphate-ribosylation factors (Arfs) are a family of Ras-related GTP binding proteins. As member of adenosine diphosphate-ribosylation factors family, Arf6 plays an important role in carcinogenesis due to its function relating to the remodelling actin cytoskeleton, cell polarity, and cell migration. The aim of this study was to compare the expression levels of *Arf6* mRNA in the tumor and adjacent healthy colon/rectum tissues in colorectal cancer patients and to determine whether there was a relationship between *Arf6* expression and the clinicopathological characteristics of patients. Tissue samples were surgically collected from 43 patients with colorectal cancer. The expression analysis of *Arf6* mRNA was evaluated by Real-Time PCR. As a result of the study, no statistically significant difference in *Arf6* expression levels was found between tumor and adjacent healthy tissues and no statistically significant relationship was found between *Arf6* expression and the clinicopathological features ($p>0.05$). The result of this study indicates that *Arf6* expression is not related to colorectal cancer.

INTRODUCTION

Colorectal cancers, which is common among digestive system cancers, rank the third among cancer-related deaths worldwide (Lin et al. 2019). It is estimated that there are over 1.8 million cases of colorectal cancer worldwide and around 880,000 colorectal cancer-related deaths in 2018 (Ciardiello et al. 2019). Colorectal cancer is a heterogeneous disease caused by the accumulation of numerous genetic and epigenetic changes (Inamura 2018; Li 2018). In addition, factors such as diet, microbiome and environmental pollutants are known to affect colorectal tumor development and progression (Cai et al. 2020). Colorectal cancer differs between genders and it is more common in men than in women, also it is 3-4 times more common in developed countries than in developing countries (Rawla et al. 2019).

[*]Address for correspondence:
Department of Biology,
Faculty of Art and Science,
Gaziantep University,
27310, Gaziantep, Turkey
Telephone: +90 532 505 13 92,
E-mail: taytekin@gantep.edu.tr

The small GTPase ADP-ribosylation factor (Arf), which is part of the Ras superfamily of small guanine triphosphate (GTP)-binding proteins, was originally defined as a co-factor that promotes cholera toxin-catalyzed ADP-ribosylation of α-subunit of the heterotrimeric G protein Gs in the 1980 (Kahn et al. 1984). Adenosine diphosphate-ribosylation factors (Arf) family of small GTPases regulate membrane traffic and membrane remodelling. In mammals, there are six isoforms of Arf GTPases (Arf 1-6). Out of these isoforms, Arf 2 was lost in humans. Arfs are divided into three classes according to their structural similarities. Arf 1-3 belong to class I, Arf 4-5 to class II, and Arf6 to class III (Liang et al. 2017). Class I and class II Arf functions particularly in the Golgi area and are involved in intracellular secretion events. Arf6 is the most diverse of the Arf isoforms and functions primarily on the cell surface (Sabe et al. 2009). In the family of adenosine diphosphate-ribosylation factors, Arf6 is a small GTPase that regulates the membrane protein traffic and endocytosis and has an important role in carcinogenesis due to its function associated with the rearrangements of the actin cytoskeleton, cell polarity and cell migration (Hashimoto et al. 2004; Morgan et al. 2015;

Liang et al. 2017; Zaoui et al. 2019). Arf6 is always expressed in different types of tissues and organs (Sabe et al. 2009).

Arf6, a member of the family of adenosine diphosphate-ribosylation factors, has an important role in the process of cancer formation due to its various functions (Hashimoto et al. 2004; Morgan et al. 2015). The relationship between Arf6 and in many cancers such as breast (Hashimoto et al. 2004), lung (Oka et al. 2014), stomach (Zhang et al. 2015), glioma (Hu et al. 2009), prostate (Morgan et al. 2015), pancreatic cancer (Taniuchi et al. 2014), melanoma (Muralidharan-Chari et al. 2009), endometrial (Zhang et al. 2020) and liver cancer cells (Qi et al. 2019) has been determined. Hashimoto et al. (2019) showed a poor relationship between the expression of Arf6 pathway components and the patient outcomes in pancreatic cancer.

Objective

In this study, *Arf6* mRNA expression levels have been investigated in colorectal cancer for the first time, and the aim was to determine whether there was a difference between tumor and normal colon/rectum tissue in patients with colorectal cancer by comparing *Arf6* mRNA expression levels. The correlation between *Arf6* expression levels and clinicopathologic parameters such as age, sex, alcohol, smoking, invasion, metastasis, and lymph node involvement were also investigated in colorectal cancer.

MATERIAL AND METHODS

Sample Collection

This study was applied onto 43 patients with colorectal cancer admitted to Gaziantep University Faculty of Medicine, Department of General Surgery. Tumors and adjacent normal colon/rectum tissue samples of these patients were collected according to ethical rules, and these tissue samples were stored at -80°C. The studied group consisted of patients who did not receive any chemotherapy or radiotherapy. Approval for the study was obtained from the Ethics Committee of Gaziantep University Faculty of Medicine.

RNA Isolation

After the homogenization of the tissue samples, RNA isolation was performed by following the protocol step which was recommended by the manufacturing company the PureLink RNA Mini Kit (Thermo Fisher Scientific, catalog no: 12183018A). The concentrations of RNA samples were determined by reading optical densities at 260 nm wavelength on the spectrophotometer. The purity of the samples was calculated by proportioning the optical density values at 260 and 280 nm wavelengths. The obtained RNA samples were stored at -80°C still further analysis.

cDNA Synthesis

cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, catalog no. 4368814) applying the Reverse Transcriptase PCR method. 2 µL 10X RT buffer, 0.8 µL 25X dNTP Mix (100 mM), 2 µL 10X RT Random primary, 1 µL Reverse transcriptase (50U/µL), 1 µL RNase inhibitor, 3.2 µL Nuclease-free water and 10 µL RNA (30 ng/µL) were used for the cDNA reaction with a total volume of 20 µL. Reaction conditions were as follows; 10 minutes at 25°C, 120 minutes at 37°C, and 5 minutes at 85°C. The cDNA samples were stored at -20°C.

Quantitative-Real Time Polymerase Chain Reaction (qRT-PCR)

For Real-time PCR reaction Taq Man Primer Probe from Thermo Fisher Scientific was used, and the used device was an Applied Biosystems (StepOne & StepOnePlus Real-Time PCR Systems). The glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene was used as the housekeeping gene. For RT-PCR, a reaction mixture with a total volume of 20 µL was prepared. This mixture included 1 µL of 20X Taq Man Gene Expression Assay (for *Arf6* or *GAPDH* genes), 10 µL 2X Taq Man Gene Expression Master Mix (Thermo Fisher Scientific, catalog no. 4369016), 7 µL RNase-free water, and 2 µL cDNA. Reaction conditions were as follows; 50°C for 2 min., 95°C for 10 min., 40 cycles of 95°C for 15 seconds, 60°C for 1 min. The experiment was per-

formed in duplicate. $2^{-\Delta\Delta Ct}$ method was used to determine expression levels (Livak and Schmittgen 2001).

Arf6 gene expression levels were measured in the tumor tissue in the patients through comparing the levels of expression in the normal tissues of the same patient. The results show how many times the gene expression level in the tumor tissue increases compared to normal tissue. $2^{-\Delta\Delta Ct}>1$ indicates increased gene expression in tumor tissue compared to normal tissue, whereas a $2^{-\Delta\Delta Ct}<1$ indicates decreased gene expression in tumor tissue. $2^{-\Delta\Delta Ct}=1$ indicates that gene expression is equal in the tumor and normal tissue (Kondo et al. 2008).

Statistical Analysis

Paired t-test was used in the comparison of the expression levels between the normal and tumor tissues, and a chi-square (χ^2) test was used to determine the relationship between clinicopathological factors grouped as categorical variable and *Arf6* gene expression levels. SPSS 22.0 software was used for analysis. *p* values less than 0.05 were accepted as statistically significant.

RESULTS

In the present study, tumor and healthy colon and rectum tissues of 43 colorectal cancer patients were studied. The mean age of the patients was 53.65 ± 0.26, out of 43 patients 29 were male, and 14 were female. Out of these patients, 60.5 percent were 50 years old and above, and 39.5 percent were under 50 years of age. 100 percent of these individuals do not use alcohol, 37.2 percent of them were smokers, and 62.8 percent were not smokers. Additionally, 23.3 percent of those colorectal cancer patients had metastases, 76.7 percent of them had not; 44.2 percent had lymph node involvement and 55.8 percent had not. Mucosal and submucosal invasion was detected in 23.3 percent of these individuals, and serosa and subserosa invasion were detected in 76.7 percent of them. 51.2 percent of the patients had no other chronic disease, and 48.8 percent had other chronic disease (Table 1).

Using Ct values, ΔCt values for each sample (normal and tumor) had been calculated. Both

Table 1: The general distribution of clinical pathologic features to the studied colorectal cancer patients

Parameters		*Patients (%)*
Age groups	50≥	26 (60.5)
	<50	17 (39.5)
Gender	Female	14 (32.6)
	Male	29 (67.4)
Smoking	-	27 (62.8)
	+	16 (37.2)
Alcohol	-	43 (100)
	+	0 (0)
Tissue type	Colon	26 (60.5)
	Rectum	17 (39.5)
Metastasis	+	10 (23.3)
	-	33 (76.7)
Invasion	I-II	10 (23.3)
	III-IV	33 (76.7)
Lymph node involvement	+	19 (44.2)
	-	24 (55.8)
Chronic disease	+	21 (48.8)
	-	22 (51.2)

ΔCt values for normal and tumor tissues were compared using the Paired t-test. The mean ΔCt value was calculated as 4.50 ± 0.46 for normal tissues and 5.00 ± 0.49 for tumor tissues. Real-Time PCR analysis showed that *Arf6* gene expression levels decreased in tumor tissue compared to normal tissue, but this difference was not statistically significant (p=0.463) (Fig. 1).

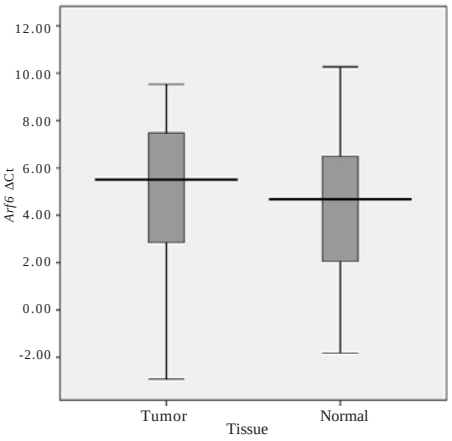

Fig. 1. Comparison of *Arf6* expression levels between normal and tumor tissues of patients with colorectal cancer

Arf6 expression levels were grouped as low or high ($2^{-\Delta\Delta CT}>1$; high, $2^{-\Delta\Delta CT}<1$; low) as a result of

the comparison of the expression in tumor tissue with the normal tissue of patients. As shown in the Table 2, no statistically significant relationship was found between *Arf6* expression levels and age (p=0.94), smoking (p=0.4), tissue type (p=0.94), metastasis status (p=0.15), invasion status (p=0.15), lymphatic invasion (p=0.2) and chronic diseases (p=0.89) of the patients with colorectal cancer. The expression level of *Arf6* was statistically higher in males than females (p=0.01).

DISCUSSION

Proliferation, invasion and metastasis of cells play a critical role in the malign transformation and tumor development. Numerous molecules are involved in malignant tumor formation, and some of these molecules have been identified. Among these molecules, Arf6, which belongs to the Ras superfamily, is known to be effective in cancer cells formation and spread due to the remodelling of actin cytoskeleton, cell polarity, cell migration, and its functions related to invadopodia (Hashimoto et al. 2004; Morgan et al. 2015; Li et al. 2017). Due to the important functions of Arf6 in carcinogenesis, the relationship between *Arf6* mRNA expression and colorectal cancer has been investigated for the first time in this study. According to the results of this study, 42 percent of patients with colorectal cancer had an increased expression of *Arf6* in tumor tissues compared to normal tissues and a decrease was observed in 58 percent of them.

Arf6 is highly expressed in breast, lung, kidney, prostate, endometrial, head and neck cancers and gastric cancer cells (Morgan et al. 2015; Hongu et al. 2017; Qui et al. 2018; Zhang et al. 2020). *Arf6* mRNA levels were found to have decreased in 90 percent of tumor tissues compared to the normal tissues of non-small cell lung cancer patients. In the same study, it was shown that Arf6 protein levels decreased in 55 percent of the same samples. Therefore, no correlation was found between protein and mRNA expressions. Consequently in the study, *Arf6* mRNA level was decreased while the amount of protein increased in tumor samples compared to normal tissues of the same individual (Knizhnik et al. 2011). In 2017, Uzozie et al. found that the expression levels of Arf6 protein decreased in colorectal adenoma samples and detected that Arf6 protein was down-regulated compared to normal mucosa in colorectal adenocarcinomas. In addition, Uzozie et al. studied the Arf6 protein level in colorectal adenocarcinomas and their results are consistent with the data obtained from our study. However, similar to breast cancer (Hashimoto et al. 2004) and non-small cell lung cancers (Knizhnik et al. 2011), an increase in Arf6 protein level may be observed while a decrease in *Arf6* mRNA is observed in tissues with colorectal cancer.

Table 2: Classification of *Arf6* expression levels to the studied colorectal cancer patients

Clinicopathological parameters		*Arf6 expression*		
		Low	*High*	*p value*
Age	<50	10	7	0.94
	≥50	15	11	
Sex	Female	12	2	0.01
	Male	13	16	
Smoking	-	17	10	0.4
	+	8	8	
Tissue type	Colon	15	11	0.94
	Rectum	10	7	
Metastatis status	-	17	16	0.15
	+	8	2	
Invasion status	I-II	8	2	0.15
	III-IV	17	16	
Lymphatic invasion	-	16	18	0.2
	+	9	10	
Chronic disease	-	13	9	0.89
	+	12	9	

It was shown that Arf6 plays an important role in the invasion of tumor cells in breast, brain, and skin cancers (Eades et al. 2015). In this current study, it was detected that 51.5 percent of the patients with advanced stage invasion had low expression levels, and the remaining 48.5 percent had high expression levels. There was no statistically significant relationship between invasion and *Arf6* mRNA expression levels. Similar to this study, no correlation was found between invasion activity and expression levels of *Arf6* mRNA in human breast cancer cell lines, but it was found that invasion activity was high in breast cancer cells with high expression levels of Arf6 protein (Hashimoto et al. 2004). Again, in 2009, Sabe et al. reported in that high expression levels of Arf6 protein in highly invasive breast cancer cells and increased transcription of Arf6 were independent of each other.

In the current study, only *Arf6* mRNA levels were determined, and no ARF6 protein level analysis was performed. It is credible to consider that the rapid transformation, degradation, and post-transcriptional modifications of mRNA molecules may have an effect on *Arf6* mRNA levels (Knizhnik et al. 2011). Post-transcriptionally controlled mRNAs encode proteins in conditions such as cell adhesion, signal transduction, growth control, and transcriptional control. Thus, the synthesis of proteins can be given both rapid and specific responses to stimuli such as stress, apoptosis, proliferation, and oncogenic stimuli through post-transcriptional control (Sabe et al. 2009). In light of these findings, it can be said that Arf is regulated by post-transcriptional mechanisms. At this point, various miRNA molecules are possible to play a role. miR-145 has been shown to affect the invasion by targeting *Arf6* mRNA in breast cancer (Eades et al. 2015) and upper tract urothelial carcinoma (Hsu et al. 2020). *Arf6* mRNA has a long 5'-untranslated region therefore, it is classified as weak mRNA (Sato et al. 2011). However, as reported in previous studies, there may not always be a match between protein and mRNA expressions (Koussounadis et al. 2015). From this point of view, studying both mRNA and protein levels can be more effective in reaching clear results in order to understand the relationship between *Arf6* mRNA, post-transcriptional control, and consequently protein level and colorectal cancer.

CONCLUSION

In this study, we investigated *Arf6* mRNA expression in colorectal cancer patients. Our results indicate that *Arf6* mRNA expression is not related to colorectal cancer according statistically analyses. Also, no relation was found between *Arf6* expression levels and clinicopathological factors.

RECOMMENDATIONS

Considering the characteristics of Arf6, it can be said that analyses both mRNA and protein levels, identification of miRNA molecules targeting *Arf6* mRNA, and determining the impact of these molecules on Arf6 will be beneficial in future studies in order to fully reveal the relationship between Arf6 and colorectal cancer. The results of our study may be limited due to the number of samples. Therefore, more samples can be studied.

ACKNOWLEDGEMENTS

This study was supported by Gaziantep University, Scientific Research Projects Governing Unit (Project No: FEF.YLT.17.08).

REFERENCES

Cai Y, Rattray NJW, Zhang Q, Mironova V, Santos-Neto A, Hsu KS, Rattray Z, Cross JR, Zhang Y, Paty PB, Khan SA, Johnson CH 2020. Sex differences in colon cancer metabolism reveal a novel subphenotype. *Sci Rep*, 10: 4905.

Ciardiello D, Vitiello PP, Cardone C, Martini G, Troiani T, Martinelli E, Ciardiello F 2019. Immunotherapy of colorectal cancer: Challenges for therapeutic efficacy. *Cancer Treat Rev,* 76: 22-32.

Eades G, Wolfson B, Zhang Y, Li Q, Yao Y, Zhou Q 2015. lincRNA-RoR and miR-145 regulate invasion in triple-negative breast cancer via targeting ARF6. *Mol Cancer Res*, 13(2): 330-338.

Hashimoto S, Furukawa S, Hashimoto A, Tsutaho A, Fukao A, Sakamura Y, Parajuli G, Onodera Y, Otsuka Y, Handa H, Oikawa T, Hata S, Nishikawa Y, Mizukami Y, Kodama Y, Murakami Y, Fujiwara T, Hirano S, Sabe H 2019. ARF6 and AMAP1 are major targets of KRAS and TP53 mutations to promote invasion, PD-L1 dynamics, and immune evasion of pancreatic cancer. *PNAS*, 116(35): 17450-17459.

Hashimoto S, Onodera Y, Hashimoto A, Tanaka M, Hamaguchi M, Yamada A, Sabe H 2004. Require-

ment for Arf6 in breast cancer invasive activities. *PNAS*, 101(17): 6647-6652.
Hongu T, Yamauchi Y, Funakoshi Y, Katagiri N, Ohbayashi N, Kanaho Y 2016. Pathological functions of the small GTPase Arf6 in cancer progression: Tumor angiogenesis and metastasis. *Small GTPases*, 7(2): 47-53.
Hsu WC, Li WM, Lee YC, Huang AM, Chang LL, Lin HH, Wu WJ, Li CC, Liang PI, Ke HL 2020. MicroRNA-145 suppresses cell migration and invasion in upper tract urothelial carcinoma by targeting ARF6. *FASEB J*, 34: 5975-5992.
Hu B, Shi B, Jarzynka MJ, Yin JJ, D'Souza-Schorey C, Cheng SY 2009. ADP-ribosylation factor 6 regulates glioma cell invasion through the IQ-domain GTPase-activating protein 1-Rac1-mediated pathway. *Cancer Res*, 69: 794-801.
Inamura K 2018. Colorectal cancers: An update on their molecular pathology. *Cancers*, 10(1): 26.
Kahn RA, Gilman AG 1984. Purification of a protein cofactor required for ADP-ribosylation of the stimulatory regulatory component of adenylate cyclase by cholera toxin. *J Biol Chem*, 259: 6228-6234.
Knizhnik AV, Kovaleva OV, Laktionov KK, Mochalnikova VV, Komelkov AV, Tchevkina EM, Zborovskaya IB 2011. Arf6, RalA, and BIRC5 protein expression in non-small cell lung cancer. *Mol Biol*, 45(2): 275-282.
Kondo I, Iida S, Takagi Y, Sugihara K 2008. MDM2 mRNA expression in the p53 pathway may predict the potential of invasion and liver metastasis in colorectal cancer. *Dis Colon Rectum*, 51(9): 1395-1402.
Koussounadis A, Langdon SP, Um IH, Harrison DJ, Smith VA 2015**.** Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Sci Rep*, 5: 10775.
Li D 2018. Recent advances in colorectal cancer screening. *Chronic Dis Transl Med* , 4(3): 139-147.
Li R, Peng C, Zhang X, Wu Y, Pan S, Xiao Y 2017. Roles of Arf6 in cancer cell invasion, metastasis and proliferation. *Life Sci*, 182: 80-84.
Liang C, Qin Y, Zhang B, Ji S, Shi S, Xu W, Liu J, Xiang J, Liang D, Hu Q, Ni Q, Yu X, Xu J 2017. ARF6, induced by mutant Kras, promotes proliferation and Warburg effect in pancreatic cancer. *Cancer Lett*, 388: 303-311.
Lin HJ, Ma XM, Wang TT, Liu Z, Man WH, Zhang ZT 2019. Clinical significance of PRR13 expression in colorectal cancer and its correlation with patients' prognosis. *Int J Hum Genet*, 19(2): 103-112.
Livak KJ, Schmittgen TD 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta CT}$ method. *Methods*, 25: 402-408.
Morgan C, Lewis PD, Hopkins L, Burnell S, Kynaston H, Doak SH 2015. Increased expression of ARF GTPases in prostate cancer tissue. *SpringerPlus*, 4: 342.
Muralidharan-Chari V, Hoover H, Clancy J, Schweitzer J, Suckow MA, Schroeder V, Castellino FJ, Schorey JS, D'Souza-Schorey C 2009. ADP-ribosylation factor 6 regulates tumorigenic and invasive properties in vivo. *Cancer Res*, 69: 2201-2209.
Oka S, Uramoto H, Shimokawa H, Yamada S, Tanaka F 2014. Epidermal growth factor receptor-GEP100-Arf6 axis affects the prognosis of lung adenocarcinoma. *Oncology*, 86: 263-270.
Qi S, Su L, Li J, Zhang C, Ma Z, Liu G, Zhang Q, Jia G, Piao Y, Zhang S 2019. Arf6-driven endocytic recycling of CD147 determines HCC malignant phenotypes. *J Exp Clin Cancer Res*, 38: 471.
Qui J, Tao L, Wei Q, Zhang P 2018. Knockdown of Arf6 increases drug sensitivity and inhibits proliferation, migration and invasion in gastric cancer SGC 7901 cells. *Oncology Lett*, 15: 2147-2152.
Rawla P, Sunkara T, Barsouk A 2019. Epidemiology of colorectal cancer: Ýncidence, mortality, survival, and risk factors. *Gastroenterology Rev*, 14(2): 89-103.
Sabe H, Hashimoto S, Morishige M, Ogawa E, Hashimoto A, Nam JM, Miura K, Yano H, Onodera Y 2009. The EGFR-GEP100-Arf6-AMAP1 signaling pathway specific to breast cancer invasion and metastasis. *Traffic*, 10: 982-993.
Sato H, Hatanaka KC, Hatanaka Y, Hatakeyama H, Hashimoto A, Matsuno Y, Fukuda S, Sabe H 2011. High level expression of AMAP1 protein correlates with poor prognosis and survival after surgery of head and neck squamous cell carcinoma patients. *J Cell Commun Signal*, 12(17).
Taniuchi K, Furihata M, Saibara T 2014. KIF20A-mediated RNA granule transport system promotes the invasiveness of pancreatic cancer cells. *Neoplasia*, 16: 1082- 1093.
Uzozie AC, Selevsek N, Wahlander A, Nanni P, Grossmann J, Weber A, Buffoli F, Marra G 2017. Targeted proteomics for multiplexed verification of markers of colorectal tumorigenesis. *Mol Cel Proteomics*, 16(3): 407-427.
Zaoui K, Rajadurai CV, Duhamel S, Park M 2019. Arf6 regulates RhoB subcellular localization to control cancer cell invasion. *J Cell Biol*, 218(11): 3812-3826.
Zhang J, Zhang Q, Sunc C, Huang Y, Zhang J, Wanga Q 2020. Clinical relevance of ARF/ARL family genes and oncogenic function of ARL4C in endometrial cancer. *Biomed Pharmacother*, 125: 110000.
Zhang Y, Du J, Zheng J, Liu J, Xu R, Shen T, Zhu Y, Chang J, Wang H, Zhang Z, Meng F, Wang Y, Chen Y, Xu Y, Gu L 2015. EGF-reduced Wnt5a transcription induces epithelial-mesenchymal transition via Arf6-ERK signaling in gastric cancer cells. *Oncotarget*, 6: 7244-7261.

Paper received for publication in December, 2019
Paper accepted for publication in June, 2020