Effects of Erythromycin on the Proliferation and Apoptosis of Cultured Nasal Polyp-Derived Cells and the Extracellular Signal-Regulated Kinase (ERK)/Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway

AE Xiaohua Liu*
CF Xin Wang*
BCG Lili Chen
EF Yuming Shi
BD Yongjia Wei

* These authors contributed equally to this work

Corresponding Author: Yongjia Wei, e-mail: zhaocheng59555@163.com

Source of support: Departmental sources

Background: Erythromycin and its derivatives have been used to treat nasal polyposis and reduce inflammation, but the mechanism of action remains unclear. The extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) pathway proteins are expressed in nasal polyps. The aim of this study was to investigate the effects of erythromycin on cell proliferation, apoptosis, and the expression of p-MEK1 and p-ERK1 on cultured nasal polyp-derived cells.

Material/Methods: Nasal polyp-derived cells (n=32) and control cells from normal inferior turbinate tissue (n=32) were divided into four groups: the control group; the erythromycin-treated (100 μM) group; the selumetinib-treated (2 nM) group; and the erythromycin + selumetinib-treated group. Western blot was used to detect p-MEK1 and p-ERK1 proteins. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect mRNA expression of BCL-2 and BAX. Flow cytometry detected expression of Ki-67 and cell apoptosis. Cell apoptosis was evaluated by terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL). Spectrophotometry assessed caspase-3 activity.

Results: The expression of Ki-67 was significantly increased, and cell apoptosis was significantly reduced in untreated nasal polyp-derived cells compared with controls. Erythromycin treatment significantly decreased cell proliferation and the expression of p-MEK1 and p-ERK1, and increased apoptosis in nasal polyp-derived cells compared with control cells. Selumetinib treatment had a synergistic effect with erythromycin to reduce the expression of p-MEK1 and p-ERK1, reduce cell proliferation, and increase cell apoptosis.

Conclusions: In cultured cells derived from nasal polyps, erythromycin treatment reduced cell proliferation and increased apoptosis by inhibiting the activation of the ERK/MAPK signaling pathway.

MeSH Keywords: Apoptosis • Erythromycin • Nasal Polyps

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/910934
Background

Nasal polyps are a common upper respiratory chronic inflammatory disease characterized by edema of the nasal mucosa, inflammatory cell infiltration, and gland hypertrophy. Currently, nasal polyps are treated by endoscopic sinus surgery, but the postoperative recurrence rate remains between 20–30% [1,2]. The mucociliary function of the sinonasal mucosa is an innate defense mechanism of the human nasal airway. Cell proliferation results in cell renewal and repair of the sinonasal mucosa. It has been shown that increased cell proliferation in nasal polyps may play an important role in the repair of the epithelial and sub-mucosal defects associated with nasal polyps [3].

A previously published study has shown that non-eosinophilic nasal polyps showed an increased change in the surface epithelium and more local maxillary involvement [4]. Changes in the dynamics in growth and proliferation in human nasal epithelial stem/progenitor cells (hNESPcs) from nasal polyps have been shown to be an important phenomenon in the pathogenesis of nasal polyps [5]. Also, the nasal epithelium plays a crucial role in innate and adaptive immune responses, and alterations in epithelial barrier function and host defense responses may contribute to the pathogenesis of chronic rhinosinusitis and nasal polyps [6]. Therefore, increased understanding of the changes of nasal polyp-derived cells may provide a molecular basis for the future diagnosis treatment of nasal polyps.

The extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) signaling pathway can activate a variety of nuclear transcription factors and participate in multiple intracellular protein kinase reactions to regulate the transcription and expression of related target genes, which affect cell biology, proliferation, apoptosis, inflammatory response, and other biological processes [7,8]. It has been shown that the expression or functional activity of key proteins in the ERK/MAPK pathway were increased in nasal polyps, suggesting that overexpression of components of the ERK/MAPK signaling pathway has a role in the pathogenesis of nasal polyps [9].

Erythromycin, a macrolide antibiotic, is widely used in clinical antibacterial therapy [10,11]. Hashiba et al. [12] observed that long-term, low-dose macrolide antibiotics have a therapeutic role in chronic sinusitis with nasal polyps. Ichimura et al. [13] found that the use of erythromycin in the treatment of chronic sinusitis and nasal polyps exerted a therapeutic effect by shrinking the polyps. Several studies have now shown that erythromycin can affect cell proliferation, apoptosis, and the release of inflammatory cytokines release through regulation of the MAPK signaling pathway [14–16]. However, the biological mechanisms for the effects of erythromycin on nasopharyngeal polyps remain unclear.

Therefore, the aim of this study was to investigate the effects of erythromycin on cell proliferation, apoptosis, and the expression of p-MEK1 and p-ERK1 and the activation of the ERK/MAPK signaling pathway on cultured nasal polyp-derived cells.

Material and Methods

Reagents and instruments

Dulbecco’s Modified Eagle’s Medium (DMEM)/F12, fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Rabbit anti-ERK1 monoclonal antibody and p-ERK1 polyclonal antibody were purchased from Abcam (Cambridge, MA, USA). Mouse anti-β-actin monoclonal antibody was purchased from Cell Signaling Technology (CST) (Beverly, MA, USA). TRNZol Universal total RNA isolation reagent was purchased from Beijing Tiangen (Beijing, China). Quantitative real-time polymerase chain reaction (qRT-PCR) reagent Transcript Green One-Step qRT-PCR SuperMix was purchased from Transgene (Beijing, China). The 5-ethyl-2’-deoxyuridine (EdU) thymidine analog cell proliferation detection kit was purchased from Molecular Probes Inc. (Eugene, OR, USA). Radioimmunoprecipitation assay (RIPA) cell lysis buffer and the terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay kit were purchased from Beyotime (Jiangsu, China). Alexa Fluor® 488 (modified fluorescein) anti-human Ki-67 and Alexa Fluor® 488 mouse IgG1 kappa isotype control antibodies were purchased from BioLegend (San Diego, CA, USA). The MEK1 inhibitor, selumetinib, was purchased from Selleckchem (Houston, TX, USA). Erythromycin was purchased from Sigma-Aldrich (St Louis, MO, USA). Biological safety cabinets and cell incubators were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Polyacrylamide gel electrophoresis (PAGE) and real-time PCR instruments were purchased from Bio-Rad (Hercules, CA, USA). The flow cytometer used in this study was purchased from Beckman Coulter (Brea, CA, USA). The inverted fluorescence microscope was purchased from Nikon (Tokyo, Japan).

Patients

Thirty-two patients with nasal polyps included 21 men and 11 women, aged between 24–52 years, who underwent their first endoscopic sinus surgery in our inpatient department between January 2016 and May 2017, were enrolled in the study. Exclusion criteria included a history of previous nasal sinus surgery, glucocorticoid or antihistamine treatment, a history of allergic rhinitis, chronic bronchitis, bronchial asthma, poor tolerance to aspirin, or other serious systemic conditions. All patients underwent unilateral or bilateral nasal computed tomography (CT) imaging with a diagnosis of nasal polyposis confirmed by histopathology, which also excluded the presence of fungal and other infectious organisms.
A further 32 patients were included as controls, who underwent endoscopic nasal sinus surgery at the Nangang Branch of Heilongjiang Provincial Hospital for deviation of the nasal septum. The control group included 22 men and 10 women, aged between 22–49 years. The inferior turbinate mucosal tissue was sampled as control tissue.

In all patients included in the study, tissue samples were collected with informed consent of the patients. The study was reviewed by the Nangang Branch of Heilongjiang Provincial Hospital Ethics Committee. The specimens were collected and stored at -80°C overnight, then transferred to liquid nitrogen for long-term storage.

TUNEL assay

The nasal polyp tissue and normal inferior turbinate mucosa tissue were sectioned for cell extraction and used to evaluate cell apoptosis according to the instructions on the TUNEL apoptosis kit. Briefly, the tissue samples were fixed in 4% paraformaldehyde for 30 min and washed twice in phosphate buffered saline (PBS). Next, the sample was incubated at room temperature for 5 min with the TUNEL assay solution containing 10% terminal deoxynucleotidyl transferase (TdT) enzyme and 90% fluorescent labeling solution. The sample was washed with PBS in triplicate, sealed with fluorescence quenching liquid, and observed under a fluorescence microscope.

Nasal polyp cell isolation and culture

There were 32 patients with nasal polyps included in the study. The nasal polyp tissue was placed in a Petri dish and washed two or three times with PBS containing 1% streptomycin. The tissue was cut into pieces and digested in DMEM/F12 medium containing 0.1% type IV collagenase at 37°C for 2h. After filtering using a 100 μm cell strainer, the solution was transferred to a 50 mL centrifuge tube and centrifuged at 300×g for 10 min. The cell pellet was resuspended in DMEM/F12 medium containing 10% FBS and placed into a 10 cm cell culture dish at 37°C and incubated in an incubator containing 5% CO2. The culture was divided into four groups: the control group; the erythromycin-treated (100 μM) group; the selumetinib-treated (2 nM) group; and the erythromycin + selumetinib-treated group. The cells were collected after 72 h of treatment. Cell proliferation was measured by the EdU assay, cell apoptosis was evaluated by flow cytometry, caspase-3 activity was assessed by spectrophotometry, and protein expression was detected by Western blot. Quantitative real-time polymerase chain reaction (qRT-PCR) was used for the detection of expression of mRNA for the anti-apoptotic factor BCL-2 and the pro-apoptotic factor BAX.

Detection of Ki-67 expression

The cells from the digested tissue were fixed with 80% methanol for 5 min and permeabilized using 0.1% PBS-Tween for 20 min. Then, the cells were incubated in the primary anti-human Ki-67 antibody, in the dark, at room temperature for 30 min and washed twice with PBS. The cells were analyzed using a Coulter FC500 MCL flow cytometer.

Nasal polyp-derived cell grouping and treatment

The isolated nasal polyp cells in the logarithmic phase were divided into four groups: the control group; the erythromycin-treated (100 μM) group; the selumetinib-treated (2 nM) group; and the erythromycin + selumetinib-treated group. The cells were collected after 72 h of treatment. Cell proliferation was measured by the EdU assay, cell apoptosis was evaluated by flow cytometry, caspase-3 activity was assessed by spectrophotometry, and protein expression was detected by Western blot. Quantitative real-time polymerase chain reaction (qRT-PCR) was used for the detection of expression of mRNA for the anti-apoptotic factor BCL-2 and the pro-apoptotic factor BAX.

Western blot

The tissue and cells were lysed in RIPA buffer at 4°C for 30 min. After centrifuging at 10000×g for 10 min, the supernatant was transferred to a new Eppendorf tube. A total of 50 μg of protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (40 V, 300 min) and transferred to polyvinylidene fluoride (PVDF) membranes (250 mA, 120 min). After blocking with 5% dried skimmed milk powder at room temperature for 60 min, the membrane was incubated with primary antibody overnight at 4°C (p-ERK1, 1: 2000) (p-MEK1, 1: 2000) (β-actin, 1: 10000). The membranes were washed three times with PBST and then incubated with horse-radish peroxidase (HRP)-labeled secondary antibody (1: 2000) for 60 min at room temperature. The membrane was washed three times with PBST, and the enhanced chemiluminescence (ECL) substrate was added at room temperature for between 2–3min. Finally, the membrane bands were scanned.

Detection of cell apoptosis

The cells were enzymatically digested and resuspended in binding buffer. Then, 5 μL Annexin-V conjugated with fluorescein isothiocyanate (FITC) and 5 μL propidium iodide (PI) were...
added to the cells, and then analyzed using a Coulter FC500 MCL flow cytometer.

**Cell proliferation assay**

A Click-iT EdU Alexa Fluor® 488 Flow Cytometry Assay Kit was used to test cell proliferation. The cells were incubated in 10 μM EdU for 2 h and then divided into the four treatment groups: the control group; the erythromycin-treated (100 μM) group; the selumetinib-treated (2 nM) group; and the erythromycin + selumetinib group. After 48 h incubation, the cells were digested with a reaction solution containing Alexa Fluor® 488 at was added at room temperature, in the dark, for 30 min. The cells were examined using a Coulter FC500 MCL flow cytometer.

**Detection of caspase-3 activity using a colorimetric spectrophotometry assay**

Caspase-3 activity was evaluated according to the manufacturer’s instructions. A pNA calibration curve was used to evaluate the 96-well plate Spectrophotometry assay and to calibrate the A405 value. The cells were seeded in 96-well plate and incubated with the Ac-DEVD-pNA spectrophotometry substrate for caspase-3 (CPP32) at 37°C for 2 h. Finally, the activity of caspase-3 was evaluated at A405 using a microplate reader.

**Statistical analysis**

All data analysis was performed using SPSS version 18.0 software. The measurement data were shown as the mean ± standard deviation (SD) and comparisons were made using a t-test. A P-value <0.05 was considered to be statistically significant.

### Results

**Cell proliferation and apoptosis in nasal polyp-derived cells**

Flow cytometry showed that Ki-67 expression in nasal polyp-derived cells was significantly increased compared with the cells from normal inferior turbinate tissues (Figure 1A), which suggested that increased cell proliferation might be involved in the pathogenesis of nasal polyps. The terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay showed that the apoptotic rate in nasal polyp-derived cells was 3.5%, which was significantly lower than that of normal inferior turbinate-derived cells, at 8.2% (Figure 1B).

**Extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) signaling pathway activation and anti-apoptotic factor expression in nasal polyp-derived cells**

Quantitative real-time polymerase chain reaction (qRT-PCR) showed that the expression of anti-apoptotic factor BCL-2 mRNA was increased, while the expression of pro-apoptotic factor BAX mRNA was decreased in nasal polyp cells compared with normal inferior turbinate cells (Figure 2A). Western blot showed that the expressions of p-MEK1 and p-ERK1 in cells from nasal polyps were increased compared with cells from normal inferior turbinate tissue, indicating that the activity of ERK/MAPK pathway was enhanced in nasal polyps (Figure 2B).
Erythromycin significantly inhibited the proliferation of cells derived from nasal polyps and induced cell apoptosis in vitro

The 5-ethynyl-2’-deoxyuridine (EdU) staining showed that the EdU positive rate of nasal polyp cells in the erythromycin-treated group was significantly lower when compared with the untreated group and was dose-dependent (Figure 3A). Flow cytometry showed that erythromycin treatment was significantly associated with apoptosis of nasal polyp cells and was dose-dependent (Figure 3B).

Erythromycin reduced nasal polyp-derived cell proliferation and induced cell apoptosis through inhibition of the ERK/MAPK signaling pathway

Western blot showed that 100 μM erythromycin significantly downregulated the expression of p-MEK1 and p-ERK1 in nasal polyp cells. Also, the MEK1 inhibitor selumetinib significantly inhibited the expression of p-MEK1 and p-ERK1 and showed a synergistic effect with erythromycin in reducing the activity of the ERK/MAPK signaling pathway (Figure 4A). EdU staining showed that selumetinib further enhanced the inhibitory effect of erythromycin on the proliferation of nasal polyp cells (Figure 4B). Flow cytometry demonstrated that the combination of selumetinib and erythromycin promoted the rate of apoptosis of nasal polyp cells compared with selumetinib or erythromycin treatment alone (Figure 4C). Spectrophotometry showed that the activity of caspase-3 in nasal polyp cells treated with selumetinib and erythromycin was significantly increased when compared with treatment with selumetinib or erythromycin alone (Figure 4D).

Discussion

Nasal polyps are a common upper respiratory tract chronic inflammatory condition characterized by a high degree of nasal mucosal edema. Nasal polyps mainly affect adults and can be located in the maxillary sinus, ethmoid, middle nasal meatus, and middle turbinate. The prevalence rate of nasal polyposis in adults has been reported to be between 1–2% [17,18]. Nasal polyps are also associated with the occurrence of other respiratory diseases, including asthma, pneumonia, and bronchiectasis.

Cell proliferation and apoptosis are regulated by multiple molecules and mechanisms that regulate the physiological or pathological growth of tissues and organs. The aberrant dynamic balance of cell proliferation and apoptosis leads to the occurrence of many diseases, such as polyps [19,20], precancerous lesions [21], and tumors [22]. In the present study, the finding that apoptosis and cell proliferation of cells derived from untreated nasal polyp tissue was increased has been supported by a previous study [3].

The mitogen-activated protein kinase (MAPK) signaling pathway is present in many eukaryotic cells. MAPK can be activated by a variety of extracellular stimuli, including cytokines, growth factors, neurotransmitters and G protein-coupled receptor ligands, through intracellular receptor tyrosine kinases, G-protein coupled receptors, and cytokine receptors [7,8]. The extracellular signal-regulated kinase (ERK)–mediated MAPK signaling pathway is a classical and major pathway, and Ras/Raf/MEK/ERK is the main transduction mode for ERK/MAPK signaling [23,24]. Recent studies have shown that interleukin
Erythromycin is an antibiotic that is commonly used to treat a wide range of bacterial infections, including respiratory tract infections, skin infections, chlamydia infections, pelvic inflammatory disease, and syphilis. Erythromycin, combined with spiramycin, has been shown to inhibit the proliferation of human mononuclear cells [26]. Also, erythromycin, and its derivatives, have been shown to inhibit the proliferation of T lymphocytes by inhibiting NF-kappaB mRNA and protein expression [27]. However, the biological and molecular mechanisms by which erythromycin can affect nasal polyp-derived cells remain unclear. In this study, nasal polyp cells were isolated and cultured in vitro. The results of this preliminary study showed that proliferation of nasal polyp-derived cells was inhibited by erythromycin in a dose-dependent manner, which was consistent with the findings from previously published studies [26,27].

Previous studies have also shown that erythromycin downregulated MAPK activity and inhibited HIV-1 replication, indicating that erythromycin has a role in regulating the MAPK signaling pathway and cell proliferation [14–16]. In the present study, erythromycin significantly downregulated the expression of p-MEK1 and p-ERK1 in nasal polyp-derived cells, whereas the ERK/MAPK pathway inhibitor selumetinib showed a synergistic effect with erythromycin to reduce cell proliferation and promote cell apoptosis; erythromycin inhibited the proliferation of nasal polyp-derived cells and induced apoptosis by suppressing the activation of the ERK/MAPK signaling pathway.

Pace et al. [28] found that the expression of the inflammatory cytokine IL-19 in nasal polyps was significantly increased compared with normal controls and that increased IL-19 expression promoted cell proliferation. The findings from this previously published study indicated that inflammation may be involved in the pathogenesis of nasal polyps [28]. Kim et al. [29] showed that Ki-67 expression and the proliferation index of epithelial cells in non-eosinophilic nasal polyps with lower airway inflammation were markedly increased compared with eosinophilic nasal polyps, indicating that in addition to inflammation, abnormal proliferation of epithelial cells is also a major factor in the pathogenesis of nasal polyps. In a previously published study that used human nasal epithelial cells in an in vitro model, lactoferrin was shown to interact with SPLUNC1 to reduce

Figure 3. Erythromycin significantly inhibited nasal polyp-derived cell proliferation and induced cell apoptosis in vitro. (A) Cell proliferation detected by 5-ethynyl-2'-deoxyuridine (EdU) thymidine analog staining. (B) Cell apoptosis detected by flow cytometry. * P<0.05, compared with the control.
lipopolysaccharide-induced inflammation of human nasal epithelial cells by downregulating the MEK1/2-MAPK signaling pathway [30]. Oda et al. [16] demonstrated that erythromycin inhibited toxin-induced TNF-α synthesis and secretion by inhibiting the activity of the ERK/MAPK pathway in neutrophils, in a similar manner to the ERK1/2 inhibitor, PD98059.

The findings of the present study, together with the findings of previously reported studies, support the potential role of erythromycin in the regulation of inflammation associated with nasal polyps and inhibit cell proliferation by inactivating ERK/MAPK signaling. Future studies are needed to further investigate the role of erythromycin, and its derivatives, on inflammation, as well as to determine the role of alternative pathways, such as NF-kappaβ, involved in the regulatory effects of erythromycin.
Conclusions

Increased activation of extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) signaling has been previously shown to be associated with nasal polyposis. In this in vitro study, cells were derived from nasal polyp tissue and were compared with cells derived from normal nasal tissue, following treatment with erythromycin. Erythromycin treatment reduced cell proliferation and increased apoptosis by inhibiting the activation of the ERK/MAPK signaling pathway.

Conflict of Interest

None.

References:

1. Fountain CR, Mudd PA, Ramakrishnan VR et al: Characterization and treatment of patients with chronic rhinosinusitis and nasal polyps. Ann Allergy Asthma Immunol, 2013; 111: 337–41
2. Jafari A, DeConde AS: Outcomes in medical and surgical treatment of nasal polyps. Adv Otorhinolaryngol, 2016; 79: 158–67
3. Hsu MC, Shun CT, Liu CM: Increased epithelial cell proliferation in nasal polyps. J Formos Med Assoc, 2002; 101: 227–29
4. Kim DK, Jin HR, Eun KM et al: Non-eosinophilic nasal polyps shows increased epithelial proliferation and localized disease pattern in the early stage. PLoS One, 2015; 10: e0139945
5. Yu XM, Li CW, Chao SS et al: Reduced growth and proliferation dynamics of nasal epithelial stem/progenitor cells in nasal polyps in vitro. Sci Rep, 2014; 4: 4619
6. Pace E, Scaffidi V, Di Bona D et al: Increased expression of IL-19 in the epithelium of patients with chronic rhinosinusitis and nasal polyps. Allergy, 2012; 67: 878–86
7. Pancione M, Giordano G, Parcesepe P et al: Emerging insight into MAPK and MEK1/2-ERK1/2 pathway is activated in chronic rhinosinusitis with nasal polyps. Arch Immunol Ther Exp (Warsz), 2014; 2: 217–29
8. Zhang X, Liu K, Zhang T et al: Cortactin promotes colorectal cancer cell proliferation by activating the EGFR-MAPK pathway. Oncotarget, 2017; 8: 1541–54
9. Linke R, Pries R, Konnecke M et al: The MEK1/2-ERK1/2 pathway is activated in chronic rhinosinusitis with nasal polyps. Arch Immunol Ther Exp (Warsz), 2014; 2: 217–29
10. Novak I, Kovac B: Electronic structure of antibiotic erythromycin. Spectrochim Acta A Mol Biomol Spectrosc, 2015; 138: 550–52
11. Broad J, Sanger GJ: The antibiotic azithromycin is a motilin receptor agonist in human stomach: Comparison with erythromycin. Br J Pharmacol, 2013; 168: 1859–67
12. Hashiba M, Baba S: Efficacy of long-term administration of clarithromycin in the treatment of intractable chronic sinusitis. J Formos Med Assoc, 2013; 111: 337–41
13. Ichimura K, Shimazaki Y, Ishibashi T, Higo R: Effect of new macrolide roxithromycin upon nasal polyps associated with chronic sinusitis. Auris Nasus Larynx, 1996; 525: 73–78
14. Zhou X, Gu D, Hou G: Erythromycin attenuates metalloprotease/anti-metalloprotease imbalance in cigarette smoke-induced emphysema in rats via the mitogen-activated protein kinase/nuclear factor-kappaB activation pathway. Mol Med Rep, 2017; 15: 2983–90
15. Komuro L, Sunazuka T, Akagawa KS et al: Erythromycin derivatives inhibit HIV-1 replication in macrophages through modulation of MAPK activity to induce small isoforms of C/EBPbeta. Proc Natl Acad Sci USA, 2008; 105: 12509–14
16. Oda M, Kihara A, Yoshioka H et al: Effect of erythromycin on biological activities induced by clostridium perfringens alpha-toxin. J Pharmacol Exp Ther, 2008; 327: 934–40
17. Chaaban MR, Walsh EM, Woodworth BA: Epidemiology and differential diagnosis of nasal polyps. Am J Rhinol Allergy, 2013; 27: 473–78
18. Willson TJ, Naclerio RM, Lee SE: Monoclonal antibodies for the treatment of nasal polyps. Immunol Allergy Clin North Am, 2017; 37: 357–67
19. Antunes A Jr, Andrade LA, Pinto GA et al: Is the immunohistochemical expression of proliferation (Ki-67) and apoptosis (Bcl-2) markers and cyclo-oxygenase-2 (COX-2) related to carcinogenesis in postmenopausal endometrial polyps? Anal Quant Cytopathol Histopathol, 2012; 34: 264–72
20. Shin JM, Byun JY, Baek BJ, Lee YJ: Cellular proliferation and angiogenesis in nasal polyps of young adult and geriatric patients. Int Forum Allergy Rhinol, 2015; 5: 41–46
21. Choy B, Lalonde A, Que J et al: MCM4 and MCM7, potential novel proliferation markers, significantly correlated with Ki-67, Bmi1, and cyclin E expression in esophageal adenocarcinoma, squamous cell carcinoma, and precancerous lesions. Hum Pathol, 2016; 57: 126–35
22. Zhang B, Guo DD, Zheng YJ, Wu YA: Expression of KLF6-SV2 in colorectal cancer and its impact on proliferation and apoptosis, Eur J Cancer Prev, 2017; 27: 20–26
23. Wang Y, Nie H, Zhao X et al: Bicyclol induces cell cycle arrest and autophagy in HepG2 human hepatocellular carcinoma cells through the PI3K/AKT and Ras/Raf/MEK/ERK pathways. BMC Cancer, 2016; 16: 742
24. Xin L, Ma X, Xiao Z et al: Cosasacckievirus B3 induces autophagy in HeLa cells via the AMPK/MEK/ERK and Ras/Raf/MEK/ERK signaling pathways. Infect Genet Evol, 2015; 36: 46–54
25. Wang Z, Li P, Zhang Q et al: Interleukin-1 beta regulates the expression of glucocorticoid receptor isoforms in nasal polyps in vitro. PLoS One, 2015; 10: e0139945
26. Roche Y, Gouveret-Pocidalo MA, Fay M et al: Macrolides and immunity: Effects of erythromycin and spiramycin on human mononuclear cell proliferation. J Antimicrob Chemother, 2013; 67: 962–70
27. Chen Y, Wang H, Tian L et al: Immunomodulatory effects of erythromycin and its derivatives on human T-lymphocyte in vitro. Immunopharmacol Immunotoxicol, 2007; 29: 587–96
28. Pace E, Scaffidi V, Di Bona D et al: Increased expression of IL-19 in the epithelium of patients with chronic rhinosinusitis and nasal polyps. Allergy, 2012; 67: 878–86
29. Kim DK, Jin HR, Eun KM et al: Non-eosinophilic nasal polyps shows increased epithelial proliferation and localized disease pattern in the early stage. PLoS One, 2015; 10: e0139945
30. Tsou YA, Tung YT, Wu TF et al: Lactoferrin interacts with SPLUNC1 to attenuate lipopolysaccharide-induced inflammation of human nasal epithelial cells via down-regulated MEK1/2-MAPK signaling. Biochim Cell Biol, 2017; 95: 394–99