ORIGINAL ARTICLE

JOURNAL OF
Obstetrics and Gynecological
INVESTIGATIONS

J Obstet Gynecol Investig 2022; 5: e1–e6
DOI: https://doi.org/10.5114/jogi.2022.114941

Submitted: 28 September 2021
Accepted: 27 February 2022

The association between low number of T regulatory cells, glycoprotein A repetitions predominant (GARP) expression, and short cervical length in preterm labour – results of a pilot study

Hana Hrbáčková[1], Michal Koucký[1], Helena Kopřivová[1,2], Karin Černá[2], Václav Čapek[3], Tereza Cindrová-Davies[4]

[1]Department of Gynaecology and Obstetrics of the First Faculty of Medicine and General Teaching Hospital, Charles University, Prague, Czech Republic
[2]Institute of Medical Biochemistry and Laboratory Diagnostics of the First Faculty of Medicine and General Teaching Hospital, Charles University, Prague, Czech Republic
[3]National Institute of Mental Health, Prague, Czech Republic
[4]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

Abstract

Introduction: Glycoprotein A repetitions predominant (GARP) regulates the function of T regulatory cells. The aim of the study was to evaluate the relationship between the T regulatory cells (Treg) numbers, GARP expression and the incidence of preterm labour in women with a short cervix.

Material and methods: Fifty-nine pregnant patients were recruited at 20–35 weeks of gestation. The control group included 29 patients who had uncomplicated pregnancies, delivered at term. Thirty patients with singleton pregnancies were identified as being at risk of preterm birth, based on a short cervical length (< 25 mm) (with or without contractions).

Results: The levels of Tregs were significantly lower in patients with a short cervix compared to controls. In addition, the GARP expression was significantly reduced in women with a short cervix who delivered at term, but not in women with a short cervix who delivered preterm.

Conclusions: There was a positive correlation between the length of the cervix and the overall duration of pregnancy. Patients with a short uterine cervix had significantly lower levels of regulatory T cells, and those who delivered at term also had reduced levels of GARP. Despite the conflicting results, our work suggests a possible role for GARP in the mechanism of preterm labour.

Key words: T cell receptor, glycoprotein A repetitions predominant, T regulatory cells, preterm labour, short cervix.

Corresponding author: Michal Koucký MD, PhD, Department of Gynaecology and Obstetrics of the First Faculty of Medicine and General Teaching Hospital, Charles University, Prague, Czech Republic, e-mail: michalkoucky@seznam.cz

To cite this article: Hrbáčková H, Koucký M, Kopřivová H, Černá K, Čapek V, Cindrová-Davies T. The association between low number of T regulatory cells, glycoprotein A repetitions predominant (GARP) expression, and short cervical length in preterm labour – results of a pilot study. J Obstet Gynecol Investig 2022; 5: e1–e6. DOI: https://doi.org/10.5114/jogi.2022.114941

Introduction

Spontaneous preterm labour complicates approximately 10% of all pregnancies. It carries the risk of children's long-term disability, posing a serious medical, social and economic problem. The most important trigger mechanisms include infections, uteroplacental ischaemia and decidual haemorrhage [1]. Preterm labour is considered to be a chronic process involving, among other things, the premature remodelling of the uterine cervix, which may lead to its shortening [2, 3]. These changes usually take place over a long time. A short cervix (< 25 mm) is a subclinical indicator that carries a significantly higher risk of preterm labour [4]. The cervical shortening is often caused by premature ripening due to subclinical infection, local inflammation, hormonal action, or genetic predisposition [5, 6]. However, cervical length measurement is not a standard screening method. Short cervical length is often an accidental finding, or it is detected by a targeted examination of patients with a history of preterm labour. Most women with a short cervix give birth at term. Therefore, there is an intensive search for other parameters that could specify the risk of preterm labour. Immune changes are likely to play a significant role in this process. Maternal immune tolerance and the mutual reciprocal communication between the maternal immune system and the developing fetus, the feto-maternal immune cross-talk, plays an essential role in establishing a successful pregnancy outcome and term delivery. The specific immune response involved in the setting and maintenance of the immunotolerant state is mediated by B and T lymphocytes, and NK cells. In particular, T regulatory cells (Tregs) play an essential role in suppressing excessive immune responses and thus providing the mechanism of tolerance for both paternal and self-antigens. Fetal tolerance is achieved by the mother's immune system without weakening of her own immune integrity. Tregs are crucial in maintaining this balance during the course of pregnancy [7]. Treg cells, a subpopulation of $CD4^+$ T lymphocytes, can limit the immune response against self and non-self antigens [8, 9] and graft rejection [10]. We have reported reduced Treg cell expression in women with preterm labour [11]. Successful pregnancy is associated with an increase in the number of Tregs in the decidua [12–15]. Treg cells express a wide range of protein markers, which are likely to play a role in modulating their function, in particular the transcription factor forkhead box P3 (FoxP3), which can suppress the transcription of key genes and thereby regulate the development and function of Tregs upon activation, and Helios, which is not expressed by naïve Treg [16]. In addition, Treg cells also express the following surface markers: CD25, latency associated peptide (LAP), glycoprotein A repetitions predominant (GARP), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), inducible co-stimulator (ICOS), tumour necrosis factor receptor 2 (TNFR2), glucocorticoid-induced tumour necrosis factor receptor (GITR), lymphocyte activation gene 3 (LAG-3), OX40 and 4-1BB. All of these proteins appear to be involved in the modulation of the bioimmune response following activation [17]. GARP is a 662 amino acid transmembrane protein, γ cell types, specifically expressed on $FoxP3^+$ T cells, and it could thus serve as an activated Treg cell surface marker [18]. GARP binds the latent transforming growth factor β (TGF-β), thereby promoting release of the biologically active TGF-β1, and facilitating Treg regulatory functions. TGF-β1 is synthesised as a precursor, which is transformed into latent non-active TGF-β1 heterodimers, Cter dimer (pre-TGF-β1), and an N-terminal latency-associated peptide (LAP). Human Treg cells form latent TGF-β1 only in association with the glycoprotein GARP via disulfide bonds between two cysteines on the GARP monomer and a cysteine on each of the LAP monomers [19]. The GARP-latent-TGF-β1 complex can be activated by factors such as matrix metalloproteinases and integrin molecules, and GARP is significantly expressed by activated Tregs to yield the biologically active TGF-β1 cytokine. Thus GARP may be, to some extent, considered an activating Treg surface marker [20]. GARP-positive Tregs are not only a source of TGF-β1 but also of interleukin (IL) 10 (IL-10). Downregulation of GARP on Treg cells decreases the expression of other Treg markers, such as CD27, CD83 and Foxp3. The GARP-latent-TGF-β1 complex induces proliferation and differentiation of the Th17 lymphocyte subpopulation. These data suggest that GARP could be considered a marker of the functional activity of Treg cells [18, 21, 22].

The aim of this study was to evaluate the relationship between the number of Treg cells and the expression of the Treg activation marker GARP and the incidence of preterm labour in women with a short cervix.

Material and methods

Patients characteristics

In this prospective study, we aimed to detect absolute and relative numbers of peripheral blood regulatory T cells, and the expression of selected surface markers of these lymphocytes in pregnant women during the second and third trimester of pregnancy. Fifty-nine pregnant patients were recruited at the Department of Gynaecology and Obstetrics of the General University Hospital and the 1st Faculty of Medicine, Charles University in Prague, between October 2015 and May 2016. The study included healthy pregnant women, aged 25 to 35 years. Patients were recruited at 20–35 weeks of gestation. Gestational age was calculated according to the date of the last menstrual period and confirmed by the first-trimester ultrasound scan. The following criteria were applied to recruit patients at risk of preterm birth: singleton pregnancy, short uterine cervix (< 25 mm) with or without regular contractions. Following diagnosis, all patients with a short cervix were prescribed progesterone treatment until the end of week 36 of gestation/birth (Utrogestan, 200 mg applied vaginally at night). The efficacy and safety of vaginal progesterone administration to prevent preterm birth in asymptomatic women with a transvaginal sonographic cervical length ≤ 25 mm was reviewed and recommended from 18–24 weeks of gestation in a meta-analysis

study [23]. Exclusion criteria included: multiple pregnancy, atopic eczema, bronchial asthma, autoimmune disease, cancer. The study group consisted of 30 women with a short cervix who were subsequently followed at the Department of Obstetrics and Gynaecology of the General Teaching Hospital, 1st Medical Faculty, Charles University Prague, due to continued regular contractions and/or a short uterine cervix. Of this group, 22 patients gave birth prematurely before 35 weeks of pregnancy (preterm delivery group, PD, $n = 22$) and 8 patients gave birth at term (term delivery, TD, $n = 8$). The control group consisted of 29 patients in the corresponding weeks of pregnancy, all with a normal cervical length, who had physiological pregnancies without complications, and delivered at term (control group, CG, $n = 29$). The study was approved by the Ethics Committee of the 1st Faculty of Medicine, Charles University, Prague (No. 1162/12 S-IV). All patients were properly informed, and they signed a written informed consent form to participate in the study.

Ultrasound measurement of cervical length

Ultrasonographic measurement of the cervical length was performed by a vaginal probe using standard equipment (Acuson XP128, Mountain View, CA). All investigations were performed by certified sonographers (trained at the Fetal Medicine Centre, London, United Kingdom). Transvaginal cervical measurements were performed using the technique described by Iams *et al.* [24]. Following the assessment of the cervical length, patients were split into two groups, as described above, i.e. a short cervical length group (< 25 mm; $n = 30$) and a control group ($n = 29$).

Blood collection

Blood samples were collected from the cubital vein during recruitment, at the onset of symptoms or following detection of a short cervix. Treg analysis was performed within 6 h of collection.

Determination of T regulatory lymphocytes

Whole blood was mixed with monoclonal antibodies raised to detect cell surface markers (anti-CD45 Krome Orange (Beckman Coulter, USA); anti-CD4 Alexa Fluor 700 (eBioscience, USA), anti-CD25 PE (Beckman Coulter, USA), anti-CD127 PE-Cy7 (Beckman Coulter, USA), anti-CD45RA FITC (Beckman Coulter, USA), anti-CD279 (PD-1), APC-eFluor 780 (eBioscience, USA), anti-GARP PE-eFluor 610 (eBioscience, USA)), incubated at 4-8°C for 30 min, followed by addition of the lysing solution (EXCELLYSE I – Exbio) and deionized H_2O. The mixture was incubated at room temperature in the dark, then centrifuged to cast the supernatant. The pellet was resuspended in PBS. This was followed by permeabilization/fixation, washes in the permeabilizing solution (Foxp3/Transcription Factor Staining Buffer, eBioscience, USA), staining with anti-Foxp3 eFluor 660 monoclonal antibodies (eBioscience, USA) for 30 min, washing and resuspension in PBS. Fluorescent intensity was measured using a Navios Beckman-Coulter cytometer. The levels of T lymphocytes were determined and compared among the three groups of patients.

Statistical analysis

To analyse the association between cervical length and time of delivery, the non-parametric Spearman correlation coefficient was used. Comparisons of levels of lymphocytes between selected groups of patients were subject to the non-parametric one-sided Wilcoxon test. Analyses were performed using the R statistical package, version 3.4.4 [25].

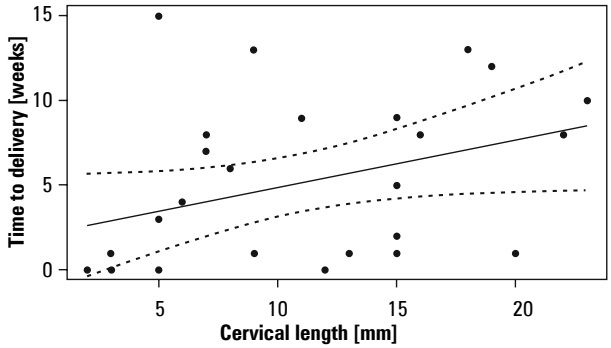
Results

Patient characteristics

Fifty-nine patients were recruited at 20–35 weeks of gestation (Table I).

Table I. Patient characteristics

Group of patients	Abbreviation	Number of patients
Control group, term delivery	CG	29
Short cervix, preterm delivery	PD	22
Short cervix, term delivery	TD	8



	Min.	1st Qu	Median	Mean	3rd Qu	Max.	SD	N	NA's
Cervical length [mm]	2.0000	5.0000	10.0000	10.8571	15.0000	23.0000	6.3463	28	0
Time to delivery [weeks]	0.0000	1.0000	4.5000	5.1071	8.2500	15.0000	4.7167	28	0

Figure 1. Time to delivery vs. cervical length. Spearman correlation coefficient $p = 0.0093$

Association between cervical length and further course of pregnancy

The cervical length was measured in all women in this study using vaginal ultrasound. In patients at risk of preterm labour (groups PD and TD) who had a short cervix (< 25 mm), there was a positive correlation between length of the cervix and length of pregnancy (Figure 1).

Absolute levels of regulatory T-lymphocytes and further development of pregnancy

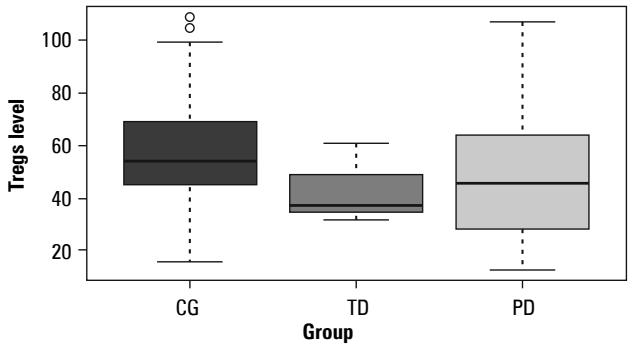
Tregs were identified as the $CD45^{+}CD4^{+}CD25^{+}CD127^{dim}FoxP3^{+}$ population of the peripheral blood T cells. Consistent with our previous findings (11), patients with a short uterine cervix (groups TD and PD) had significantly lower levels of Tregs (Figure 2).

Levels of GARP expression in peripheral blood T-lymphocytes in patients with different pregnancy outcomes

The other aim of this study was to investigate whether the GARP expression levels on Treg cells were indicative of preterm labour in patients with a short cervix. GARP expression was significantly reduced in women with a short cervix who delivered at term, compared to the controls who delivered at term. However, the GARP levels were not significantly different in the patients with a short cervix who delivered preterm. This marker is thus unlikely to be used as a preterm labour predictor (Figure 3).

Discussion

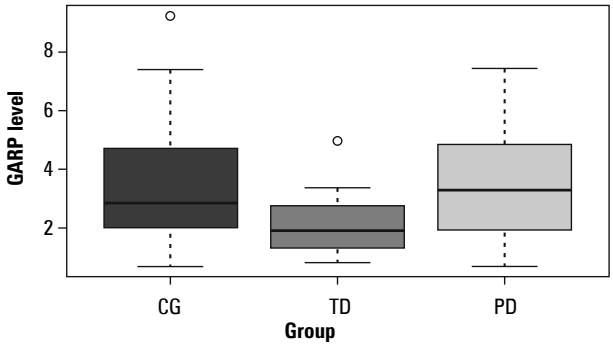
The aim of our study was to determine whether there is a difference in the total number of circulating T regulatory lymphocytes and the expression of one of their receptors, GARP, in women with a short cervix compared to a group of pregnant women with normal cervical length. Our results show a positive correlation between the length of the cervix and the duration of pregnancy. In addition, all patients with a short uterine cervix had significantly lower levels of regulatory T-lymphocytes compared to controls with normal cervical length, which is consistent with our previous findings [26]. Tregs have a key regulatory function in pregnancy –



	Min.	1st Qu	Median	Mean	3rd Qu	Max.	SD	*N*	NA's
CG	15.4700	45.1400	54.1600	58.7148	68.7900	109.4400	23.1160	29	0
TD	31.4700	34.6425	37.2300	41.8338	45.1875	60.8600	11.1016	8	0
PD	12.4300	27.9600	45.5200	46.4881	64.0100	107.3000	27.0805	21	0

	P-value
CG vs. TD	0.0176
TD vs. PD	0.4714
CG vs. PD	0.0401

Figure 2. Treg level vs. group. Treg levels are expressed as count × 10^6 per l.



	Min.	1st Qu	Median	Mean	3rd Qu	Max.	SD	*N*	NA's
CG	0.6900	2.0100	2.8700	3.6800	4.7400	9.2300	2.1763	29	0
TD	0.8300	1.3375	1.9050	2.2163	2.4350	4.9600	1.3394	8	0
PD	0.7200	1.9300	3.2800	3.5071	4.8500	7.4200	2.0956	21	0

	P-value
CG vs. TD	0.0325
TD vs. PD	0.9305
CG vs. PD	0.4298

Figure 3. GARP level vs. group. GARP levels are expressed as count × 10^6 per l.

their main task is to avoid excessive inflammatory responses. Natural Treg cells originate in the thymus after contact with autoantigens. Adaptive iTregs, which play a key role in reproduction, arise in the peripheral tissues upon contact with antigens that are not expressed in the thymus, but are specifically restricted to their respective peripheral tissues. In other words, adaptive Tregs control the response of the immune system to foreign exogenous antigens against which it is not rational to develop an aggressive immune response [26]. Antigen-stimulated Treg cells typically regulate immune responses by producing the cytokines IL-10 and TGF-β. In this study, the levels of circulating Tregs were significantly lower in women who had preterm deliveries, compared to those who delivered at term. This is in agreement with our previous findings [27]. We also aimed to investigate whether changes in GARP expression may be indicative of threatened preterm labour in subclinical patients with a short cervix. T regulatory lymphocytes and their receptors have strong immunosuppressive activity as they inhibit the anti-tumour immune response in tumour-bearing hosts [28, 29]. Some of the molecules may also have possible therapeutic significance. For this reason, regulatory molecules of various immune cell populations represent a promising target. For example, progesterone plays a very important role in immunomodulation, and it is likely to modulate GARP expression *in vitro*. We did not find significant differences between GARP expression in patients with a short cervix who delivered preterm, but we found significantly reduced GARP expression in patients with a short cervix who gave birth at term, compared to controls with normal cervical length. All patients with a short cervix were prescribed progesterone at the time of diagnosis. We assume that our results were influenced not only by low patient numbers, but also by the progesterone administration, which is known to prolong pregnancy in patients with a short cervix [23]. Progesterone exerts pleiotropic effects on immune processes in pregnancy, both systemically and locally at the maternal-fetal interface, leading to a successful continuation of pregnancy [30]. It was not possible to use the Treg level measurement as a reliable predictor of preterm delivery. In general, the prediction of preterm labour is significantly modified by the measures that come with its management. To date, very limited data are available on the role of GARP expression on Treg lymphocytes in pregnancy. Only one study reported that human neutrophils exposed to the pregnancy hormones progesterone and estriol promoted the establishment of maternal tolerance through the induction of a population of $CD4^+$ T cells displaying a $GARP^+CD127^{lo}FOXP3^+$ phenotype following antigen activation [31]. High expression of GARP on Tregs has been reported in individuals with malignant tumours who do not respond to anti-tumour therapy, and high GARP expression is associated with a higher risk of metastatic disease [32]. Although further studies are needed to explain the role of GARP in processes of fetal tolerance and pathological inflammatory mechanisms leading to preterm labour, our study demonstrates the reality of the different expression of GARP molecules on Tregs in women with preterm delivery. We are aware of the limitations of this study. We consider the fact that we evaluated the maternal circulating pool of T regulatory lymphocytes to be the main one. Evaluation of decidual T regulatory lymphocytes and their receptors would have yielded far more accurate data. However, our study design did not allow for this investigation. We can only speculate that research in this area will provide more accurate information by studying the expression of T regulatory molecules directly in the placental bed. The GARP molecule itself, as well as the GARP+ latent TGF-β1 complex, could theoretically become the target structure of the biological treatment of immunopathological inflammation associated with preterm labour, as is already contemplated for the therapy of certain tumours, autoimmune and inflammatory diseases.

In conclusion, in our study, we confirm that all patients with a short uterine cervix had significantly lower levels of regulatory T cells. This is the first study examining the expression of GARP in women with signs of preterm labour. Despite the conflicting results, our work suggests a possible role for GARP in the background of preterm labour.

Acknowledgments

This project was supported by the Research Projects PRVOUK P25/LF1/2 and UNCE No. 204024 of Charles University, Prague, and by RVO-VFN64165/2012 of the General University Hospital in Prague.

Conflict of interest

The authors declare no conflict of interest.

References

1. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O. The preterm parturition syndrome. BJOG 2006; 113 Suppl 3: 17-42.
2. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med 2007; 25: 21-39.
3. Christiaens I, Zaragoza DB, Guilbert L, Robertson SA, Mitchell BF, Olson DM. Inflammatory processes in preterm and term parturition. J Reprod Immunol 2008; 79: 50-7.
4. Hassan SS, Romero R, Berry SM, et al. Patients with an ultrasonographic cervical length < or =15 mm have nearly a 50% risk of early spontaneous preterm delivery. Am J Obstet Gynecol 2000; 2182: 1458-67.
5. Vaisbuch E, Hassan SS, Mazaki-Tovi S, et al. Patients with an asymptomatic short cervix (<or=15 mm) have a high rate of subclinical intraamniotic inflammation: implications for patient counseling. Am J Obstet Gynecol 2010; 202: 433 e1-8.
6. Crane JM, Hutchens D. Transvaginal sonographic measurement of cervical length to predict preterm birth in asymptomatic women at increased risk: a systematic review. Ultrasound Obstet Gynecol 2008; 31: 579-87.
7. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008; 133: 775-87.
8. Marson A, Kretschmer K, Frampton GM, et al. Foxp3 occupancy and regulation of key target genes during T-cell stimulation. Nature 2007; 445: 931-5.

9. Fife BT, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. Ann N Y Acad Sci 2011; 1217: 45-59.
10. Yost NP, Bloom SL, Twickler DM, Leveno KJ. Pitfalls in ultrasonic cervical length measurement for predicting preterm birth. Obstet Gynecol 1999; 93: 510-6.
11. Koucky M, Malickova K, Cindrova-Davies T, et al. Low levels of circulating T-regulatory lymphocytes and short cervical length are associated with preterm labor. J Reprod Immunol 2014; 106: 110-7.
12. Heikkinen J, Mottonen M, Alanen A, Lassila O. Phenotypic characterization of regulatory T cells in the human decidua. Clin Exp Immunol 2004; 136: 373-8.
13. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Human Reprod 2004; 10: 347-53.
14. Sanchez-Ramon S, Navarro AJ, Aristimuno C, et al. Pregnancy-induced expansion of regulatory T-lymphocytes may mediate protection to multiple sclerosis activity. Immunol Letters 2005; 96: 195-201.
15. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology 2004; 112: 38-43.
16. Ng MSF, Roth TL, Mendoza VF, Marson A, Burt TD. Helios enhances the preferential differentiation of human fetal CD4(+) naive T cells into regulatory T cells. Sci Immunol 2019; 4: eaaav5947.
17. Stockis J, Colau D, Coulie PG, Lucas S. Membrane protein GARP is a receptor for latent TGF-beta on the surface of activated human Treg. Eur J Immunol 2019; 39: 3315-22.
18. Zhou AX, Kozhaya L, Fujii H, Unutmaz D. GARP-TGF-beta complexes negatively regulate regulatory T cell development and maintenance of peripheral CD4+ T cells in vivo. J Immunol 2013; 190: 5057-64.
19. Wang R, Zhu J, Dong X, Shi M, Lu C, Springer TA. GARP regulates the bioavailability and activation of TGFbeta. Mol Biol Cell 2012; 23: 1129-39.
20. Fridrich S, Hahn SA, Linzmaier M, et al. How soluble GARP enhances TGFbeta activation. PLoS One 2016; 1: e0153290
21. Hahn SA, Neuhoff A, Landsberg J, et al. A key role of GARP in the immune suppressive tumour microenvironment. Oncotarget 2016; 7: 42996-3009.
22. Metelli A, Salem M, Wallace CH, et al. Immunoregulatory functions and the therapeutic implications of GARP-TGF-beta in inflammation and cancer. J Hematol Oncol 2018; 11: 24.
23. Conde-Agudelo A, Romero R. Vaginal progesterone to prevent preterm birth in pregnant women with a sonographic short cervix: clinical and public health implications. Am J Obstet Gynecol 2016; 214: 235-42.
24. Iams JD, Goldenberg RL, Meis PJ, et al. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med 1996; 334: 567-72.
25. Team CR. R: A language and environment for statistical computing. R Foundation for Statistical Computing [Internet]. 2018.
26. Sun L, Jin H, Li H. GARP: a surface molecule of regulatory T cells that is involved in the regulatory function and TGF-beta releasing. Oncotarget 2016; 7: 42826-36.
27. Gomez-Lopez N, Laresgoiti-Servitje E. T regulatory cells: regulating both term and preterm labor? Immunol Cell Biol 2012; 90: 919-20.
28. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci 2019; 110: 2080-9.
29. Shevach EM. GARP as a therapeutic target for modulation of T regulatory cell function. Expert Opin Ther Targets 2017; 21: 191-200.
30. Shah NM, Lai PF, Imami N, Johnson MR. Progesterone-related immune modulation of pregnancy and labor. Front Endocrinol 2019; 10: 198-210.
31. Nadkarni S, Smith J, Sferruzzi-Perri AN, et al. Neutrophils induce proangiogenic T cells with a regulatory phenotype in pregnancy. Proc Natl Acad Sci USA 2016; 113: E8415-24.
32. Jin H, Sun L, Tang L, Yu W, Li H. Expression of GARP is increased in tumour-infiltrating regulatory T cells and is correlated to clinicopathology of lung cancer patients. Front Immunol 2017; 8: 138.