Original Article

Cullin 1 as a Proangiogenic Factor and its Relationship with Vascular Endothelial Growth Factor and p21 are Associated with the Length of Gestational Age of Pre-eclampsia

Tjam Diana Samara[1,2], Heri Wibowo[1,3], Isabella Kurnia Liem[1,4], Ani Retno Prijanti[1,5], Andrijono[1,6]

[1]Department of Biomedical Science,[3]Parasitology, [4]Anatomy, [5]Biochemistry and Molecular Biology and [6]Obstetrics and Gynaecology, Faculty of Medicine, Universitas Indonesia,[2]Department of Anatomy, Faculty of Medicine, Trisakti University, Jakarta, Indonesia

Abstract

Background: Cullin 1 (CUL1), vascular endothelial growth factor (VEGF), and p21 are proteins that play a role in pregnancy. CUL1 and VEGF are proangiogenic factors, whereas p21 is an antiangiogenic factor. An imbalance between proangiogenic and antiangiogenic factors is one of the various factors that cause pre-eclampsia (PE). The aim of this study was to analyze CUL1 levels in the relationship with VEGF levels and p21 levels based on gestational age at delivery in PE. **Materials and Methods:** This was a cross-sectional study of 70 placentas from PE patients divided in two groups of gestational age at delivery: <34 weeks and ≥34 weeks. Levels of CUL1, VEGF, and p21 were examined by the enzyme-linked immunosorbent assay technique. Statistical analysis was assessed by Spearman correlation test and Chi-square test, with a significant $P < 0.05$. **Results:** Low levels of CUL1 and low levels of VEGF were related with <34 weeks of gestational age at delivery, whereas high levels of CUL1 and high levels VEGF were related with ≥34 weeks of gestational age at delivery. There was a negative correlation between the high ratios of p21/CUL1 and <34 weeks of gestational age at delivery in PE. **Conclusions:** Low levels of CUL1 and low levels VEGF and high ratios of p21/CUL1 were associated with <34 weeks of gestational age at delivery in PE.

Keywords: Cullin 1, gestational age, p21, pre-eclampsia, vascular endothelial growth factor

Introduction

The success of a pregnancy needs a balance between proangiogenic and antiangiogenic factors made by the placenta. In early pregnancy, proangiogenic factors dominate, whereas at the end of pregnancy, antiangiogenic factors take over, this is thought to be for the birth preparation.[1] Proangiogenic and antiangiogenic proteins have been thought to be related to the pathogenesis of pre-eclampsia (PE). Proangiogenic protein stimulates trophoblast invasion.[2,3] An imbalance is suspected because antiangiogenic factors appear too early and/or if the production is excessive.[1] Proangiogenic factors will increase trophoblast invasion in early pregnancy.[4] Increased excessive antiangiogenic factors early in pregnancy will cause inhibited invasion of trophoblasts, thus disrupting pseudovascularization. An imbalance of the proangiogenic and antiangiogenic factors is one of many factors can cause PE.[1]

PE is a syndrome in pregnancy disorders with etiology and pathogenesis which remain unknown yet. PE is the development of hypertension after 20 weeks of gestation or immediately after delivery with proteinuria or thrombocytopenia, renal insufficiency, liver dysfunction, lung edema, cerebral disorder, or visual impairment.[5]

Cullin 1 (CUL1) and vascular endothelial growth factor (VEGF) are proangiogenic factors, while p21 is an antiangiogenic factor, which some of the proteins play a role in pregnancy. The imbalance of between proangiogenic factors and antiangiogenic factors is the one of many etiologies could cause PE. CUL1 is

Access this article online

Quick Response Code:

Website: www.jnsbm.org

DOI: 10.4103/jnsbm.JNSBM_123_20

Address for correspondence: Dr. Tjam Diana Samara, Fakultas Kedokteran, Universitas Trisakti, Jln. Kyai Tapa Grogol, Jakarta, Indonesia.
E-mail: dianasamara@trisakti.ac.id

Submitted: 26-Jun-2020 **Revised:** 18-Aug-2020
Accepted: 09-Oct-2020 **Published:** 15-Jul-2021

How to cite this article: Samara TD, Wibowo H, Liem IK, Prijanti AR, Andrijono. Cullin 1 as a proangiogenic factor and its relationship with vascular endothelial growth factor and p21 are associated with the length of gestational age of pre-eclampsia. J Nat Sc Biol Med 2021;12:140-4.

a human protein from the Cullin family gene. It is an essential component of the SCF (SKP1-CUL1-F-box protein) E3 ubiquitin ligase complex.[6] CUL1 plays an important role in protein degradation and protein ubiquitination, mediating protein ubiquitination to be involved in progressive cell cycles, signal transduction, and transcription.[7] CUL1 is more common in the first trimester of pregnancy compared to the third trimester in a normal pregnancy. CUL1 stimulates trophoblast cell invasion.[8]

VEGF is a signaling protein that regulates endothelial cell proliferation, triggers the growth of new blood vessels (angiogenesis), and vascular permeability. Several studies had shown an increase in VEGF circulation in PE. It seems that severe vasoconstriction in PE would increase vascular shear-stress, and a reversal in the increase in VEGF circulation occurred. However, other studies showed a decrease or no change in VEGF levels in PE.[9]

p21 is a cyclin-dependent kinase inhibitor (also known as p21WAF1/Cip1). Besides being known to inhibit growth and induce senescence, p21 also inhibits apoptosis, so that its oncogenic activity seems paradoxical. p21 has a paradoxical role in apoptosis, besides being able to inhibit apoptosis, it can also be an apoptotic modulator. p21 also triggers apoptosis through p53-dependent and p53-independent mechanisms under certain cellular stress.[10] In some cases of PE, p21 expression showed a double band that was consistent with cleavage by caspase, where there was an increase in CASP3 activity in PE through increased pro-CASP3.[11] In PE, excessive expression of p21 was found.[12]

There are still no studies about the relationship between CUL1, VEGF, dan p21 pada pre-eclamptic placenta. Hence, that the aim of this study was to analyze the relationship between CUL1 and VEGF and p21 associated with the differences of gestational age at delivery in PE. There are still no studies about the relationship between CUL1, VEGF, and p21 in pre-eclamptic placenta. Hence, that the aim of this study was to analyze the relationship between CUL1 and VEGF and p21 associated with the differences of gestational age at delivery in PE. The novelty of this study was to know the relation between CUL1 and VEGF which both as proangiogenic factors and p21/CUL1 ratios which p21 as antiangiogenic factor and CUL1 as proangiogenic factor.

Materials and Methods

Patient information

This study was a cross-sectional design. PE patients above 18 years old were included in this study. Placental samples were obtained with written informed consent by the patients. PE patients with diabetes mellitus or chronic kidney failure were excluded in this study. PE was defined according to the criteria recommended by the American College of Obstetrics and Gynecologist guidelines 2013: new-onset hypertension developing after 20 weeks of gestation (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90).[5]

This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia at January 16, 2017 (N0.34/UN2.F1/ETIK/2017).

Placenta collection

Seventy placentas were obtained from PE patients through vaginal delivery or cesarean section according to the consecutive random sampling. The samples were divided into two groups: <34 weeks of gestational age (n = 26) and ≥34 weeks of gestational age (n = 44) at delivery. Placentas dissected ½ cm × ½ cm × ½ cm from the maternal side on the random area were inserted into small tubes, then processed into homogenates. It did not need sterile tissue placenta, because the measurement used enzyme-linked immunosorbent assay (ELISA) method.

Homogenates process

Tissues were rinsed in ice-cold Phosphate-buffered saline to remove excess blood thoroughly and weighed before homogenization. One hundred milligrams tissues were minced to the small pieces and homogenized them in 1 ml cold PBS with a glass homogenizer. The resulting suspension was sonicated with ultrasonic cell disrupter till the solution is clarified. All the procedures were done on ice. The homogenates were centrifuged for 5 min at 5000 ×g, 2°C–8°C. Collect the supernatants and put into micro tubes and then stored at − 80°C until the time for ELISA assay for CUL1 (catalog: MBS921353 – MyBiosource – USA), VEGF (catalog: SEA143Hu – Cloud-Clone Corp., – USA), and p21 (catalog: SEE624Hu-Cloud-Clone Corp.– USA).

Enzyme-linked immunosorbent assay

ELISA technique followed the procedure written in manual according to the product. Briefly, determine wells for diluted standard, blank, and sample. Prepare seven wells for standard, one well for blank. Add 100 µl each of dilutions of standard, blank, and supernatant into the well. Cover with the plate sealer. Incubate for 1 h at the room temperature and then remove the liquid of each well, don't wash. Add 100 µl of Detection Reagent A working solution to each well, cover the wells with the plate sealer and incubate for 1 h at the room temperature. Aspirate the solution and wash with 350 µl of once wash solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser, and let it sit for 1–2 min. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash three times. After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper. Add 100 µl of Detection Reagent B working solution each well, cover the wells with the plate sealer and incubate for 30 min at room temperature. Repeat the aspiration/wash process for total five times. Add 90 µl of substrate solution to each well. Cover with a new plate sealer. Incubate for 10–20 min at the room temperature. Protect from light. The liquid will turn blue by the addition of substrate solution. Add 50 µl of stop solution to each well. The liquid will turn yellow by the addition of stop solution. Mix the liquid by tapping the side of the plate.

If color change does not appear uniform, gently tap the plate to ensure thorough mixing. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct the measurement at 450 nm immediately. All protein results from ELISA were then calculated based on total protein levels in the placenta.

Statistical analysis

The Spearman correlation test was used because it was nonparametric data. A Chi-square test was performed to assess whether there was a relationship between the group of protein level and group of gestational age at delivery. Receiver operating characteristic (ROC) test was performed to determine cutoff point the level of CUL1, VEGF, and p21. Statistical conclusions were taken with a 95% confidence level and significant $P < 0.05$.

Results

Clinical characteristics of pre-eclampsia patients

The youngest was 19 years old, and the oldest was 44-year-old. The lowest systolic blood pressure was 130 mmHg, and the highest was 220 mmHg. The lowest diastolic blood pressure was 79 mmHg, and the highest was 150 mmHg. The lowest proteinuria was zero, and the highest proteinuria was four. CUL1 levels are lower than VEGF and p21 levels in placenta [Table 1].

Proangiogenic effect: Relationship Cullin 1 with vascular endothelial growth factor

ROC test showed that cutoff point of CUL1 levels was 17.27 pg/mg and VEGF levels was 198.57 pg/mg. The relationship between CUL1 and VEGF which was seen in its distribution based on <34 weeks and ≥34 weeks of gestational age at delivery could be seen in Table 2. There were 35 cases (50%) out of 70 cases of PE had the high levels of CUL1 and high levels of VEGF. Based on the distribution pattern [Figure 1], out of 35 cases had high levels of CUL1 and high levels of VEGF, there were nine cases (25.7%) of <34 weeks of gestational age and 26 cases (74.3%) of ≥34 weeks of gestational age at delivery.

Conversely, there were 16 cases of PE (22.9%) are found out of total cases (70 cases) had low levels of CUL1 and low levels of VEGF. Based on the low levels of CUL1 and low levels of VEGF, there were 11 cases (11/16 × 100% = 68.8%) of <34 weeks of gestational age at delivery. Whereas only five cases (31.2%) were ≥34 weeks of gestation at delivery [Table 2].

Table 1: Characteristics and levels of Cullin 1, vascular endothelial growth factor, p21 of pre-eclampsia patients

Variables	*n*	Minimum	Maximum	Median
Mothers's age (years)	70	19	44	30.5
Blood pressure				
Systolic (mmHg)	70	130	220	166.50
Diastolic (mmHg)	70	79	150	102
Proteinuria (dipstick test)	61	0	4	2
Levels of protein in placentas				
CUL1 (pg/mg)	70	2.09	93.98	28.7
VEGF (pg/mg)	70	64.29	428	215.74
P21 (ng/mg)	70	0.01	0.10	0.04

CUL1: Cullin 1, VEGF: Vascular endothelial growth factor

Antiangiogenic effect: P21/Cullin 1 ratios

ROC test showed that cutoff point of p21/CUL1 ratios was two point seven. The results of this study indicated that the

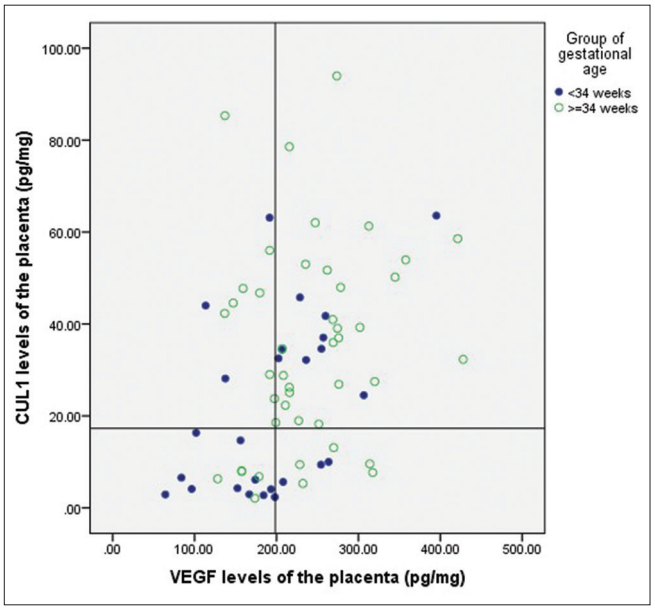

Figure 1: Distribution patterns of Cullin 1 and vascular endothelial growth factor levels based on the group of gestation age at delivery in pre-eclampsia. Note: Cut off points the levels Cullin 1 was 17.27 pg/mg in placenta and the levels of vascular endothelial growth factor was 198.57 pg/mg in placenta

Table 2: The relationship between cullin 1 and vascular endothelial growth factor levels in pre-eclampsia

Gestational age at delivery	CUL1 and VEGF levels				Total (%)	*P* Chi-square test
	LL (%)	LH (%)	HH (%)	HL (%)		
<34 weeks	11 (68.8)	3 (37.5)	9 (25.7)	3 (27.3)	26 (37.1)	0.005*
≥34 weeks	5 (31.2)	5 (62.5)	26 (74.3)	8 (72.7)	44 (62.9)	
Total	16 (100)	8 (100)	35 (100)	11 (100)	70 (100)	

LL: Low levels of CUL1 and VEGF, LH: Low levels of CUL1 and high levels of VEGF, HH: High levels of CUL1 and VEGF, HL: High levels of CUL1 and low levels of VEGF (low levels of CUL1: <17.27 pg/mg; high levels of CUL1: ≥17.27 pg/mg; low levels of VEGF: <198.57 pg/mg; high levels of VEGF: ≥198.57 pg/mg). *Significant $P<0.05$. CUL1: Cullin 1, VEGF: Vascular endothelial growth factor

antiangiogenic effect on placental tissue in PE cases would be significant if ratio-based analysis was performed. There was a negative correlation between p21/CUL1 ratios and <34 weeks of gestational age ($R = -0.481$; $P = 0.006$), and there was no correlation between p21/CUL1 ratios and ≥34 weeks of gestational age ($R = -0.036$; $P = 0.409$) [Figure 2].

Based on the Chi-square test, it was found a significant relationship between p21/CUL1 ratios and gestational age of delivery at PE. There were 13 cases (61.9%) of all high ratios of p21/CUL found at <34 weeks of gestational age at delivery. Whereas low ratios of p21/CUL1 were 36 cases (73.5%) of all low ratios of p21/CUL1 found at ≥34 weeks of gestational age at delivery [Table 3]. Low and high ratios of the p21/CUL1 were performed by the ROC test (results in Appendix).

Discussion

In this study, gestational age was divided into two groups, namely <34 weeks and ≥34 weeks at delivery. The age limit of 34 weeks' gestation was based on the general division of early onset PE (EOPE) and late onset PE (LOPE). The incidence of EOPE (gestational age <34 weeks) has a different etiology than LOPE (gestational age ≥34 weeks), which causes different complications.[13-16]

Proangiogenic effect: Cullin 1 and vascular endothelial growth factor

In this current study, based on the results of the Chi-square test [Table 2] and the pattern of distribution of CUL1 and VEGF levels [Figure 1] showed that the frequency of high levels both of CUL1 and VEGF were found in ≥34 weeks of the gestational age more than <34 weeks of gestational age at delivery. Meanwhile, the frequency of low levels CUL1 and low levels VEGF were found in <34 weeks of the gestational age more than ≥34 weeks of gestational age at delivery. Based on these results, it could be concluded that CUL1 and VEGF as proangiogenic proteins played an important role in gestational age at delivery. As Zhang *et al*.[8] found that the level of CUL1 protein in human placental villus from PE patients was significantly lower compared to the equivalent control placenta.

In addition, the expression of CUL1 in the placenta at term pregnancy was significantly lower than in the early trimester pregnancy with Western blot test. The results indicated the role of CUL1 on the ability of trophoblast invasion.[8] VEGF also has an important role in the regulation of the trophoblast invasion, proliferation, and differentiation during pregnancy. VEGF is identified the villous cytotrophoblast in the first trimester, meanwhile VEGF is found in the syncytiotrophoblast and extravillous in the term placenta.[17] Sezer *et al*.[18] found that VEGF levels in the placenta of PE patients lower than their levels in placenta of normal pregnancy.

It is known that trophoblast invasion requires a proangiogenic factor, so if the levels of the proangiogenic factors were low in early gestational age, it would make the pregnancy worse and could not be maintained. Besides, there is still no study that correlates between CUL1 and VEGF in PE. Therefore, further study is needed to find out the levels of proangiogenic proteins that affect pregnancy especially in PE.

Antiangiogenic effect: P21/Cullin 1 ratios

There is an increased in levels of p21 in normal matured placentas.[12] p21 is known to inhibit growth and induce senescence and can also be an apoptotic modulator.[10] However, if there is an excessive increase in senescence, there would cause PE. Thus, it was found excessive expression of p21 in PE.[12] The results of this study indicated that the p21/CUL1 ratios was negatively correlated with <34 weeks of gestational age but did not correlate with >34 weeks of gestational age at delivery in PE. This result showed that in <34 weeks of gestational age at delivery

Table 3: The relationship between p21/cullin 1 ratios and gestational age at delivery in pre-eclampsia

Gestational age at delivery	p21/CUL1 ratios			*P*
	Low <2.7(%)	High ≥2.7(%)	Total(%)	Chi-square test
<34 weeks	13 (26.5)	13 (61.9)	26 (37.1)	0.005
≥34 weeks	36 (73.5)	8 (38.1)	44 (62.9)	
Total	49 (100)	21 (100)	70 (100)	

According ROC test: cutoff point significant $P<0.05$. CUL1: Cullin 1, ROC: Receiver- operating characteristic

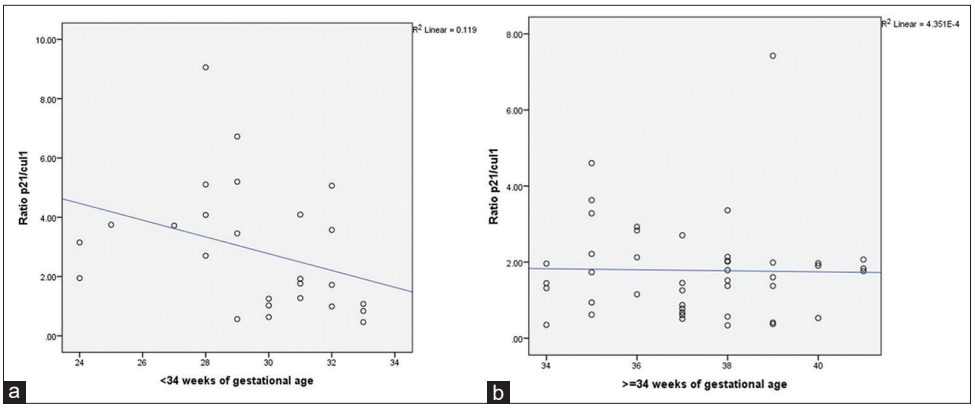

Figure 2: The correlation between p21/Cullin 1 ratios and gestation age at delivery in preeclampsia. (a) <34 weeks of gestational age at delivery ($R = -0.481$; $P = 0.006$). (b) ≥34 weeks of gestational age at delivery ($R = -0.036$; $P = 0.409$). Note: significant $P < 0.05$

in PE, the lower gestational age followed by the higher ratios of p21/CUL1. The high ratios mean that there was a possibility that levels of p21 increased and levels of CUL1 was normal or levels of p21 was normal and levels of CUL1 decreased, or levels of p21 decreased and levels of CUL1 decreased much more. This situation was also seen in a significant relationship between p21/CUL1 ratios and gestational age group. Low ratios of p21/CUL1 were found at ≥34 weeks of gestational age, whereas high ratios of p21/CUL1 were found at <34 weeks of gestational age at delivery in PE. These results indicated that if the levels of the antiangiogenic group (p21) increased and the levels of the proangiogenic group (CUL1) decreased, the gestational age at delivery tended to be earlier in <34 weeks of the gestational age group. This result showed that p21 as antiangiogenic factor had excessive expression in PE.[12]

It was known that MLN4924 completely inhibits cullin neddylation, inactivated CRL (Cullin Ring Ligase), and caused accumulation of CRL substrates, including p21. This result was proven by Li *et al*.[19] in a study of lung cancer patients. Similar results were also found by Wang *et al*.[20] in prostate cancer patients; therefore increasing CUL1 would make p21 levels decrease and vice versa. Meanwhile, placental CUL1 is not secreted into maternal blood,[21] therefore p21/CUL1 ratios cannot be used as a marker for examination in maternal blood, whereas biopsy of the placenta is not recommended because it carries a high risk. Besides, there is still no study that correlates between p21/CUL1 ratios and gestational age at delivery in PE. Further study is needed to find out the factors that influence CUL1 and p21. Based on the results of the current study, it needs for clinicians to consider about the factors or supplements that can help the balancing of angiogenic factors in pregnancy women.

The limitation of this study was the sample collections were not taken in the same location of the maternal side of the placenta. There is no control which match gestational age of normal pregnancy patients were not examined for comparison with PE.

Conclusions

Low levels of CUL1 were accompanied with the low levels of VEGF and high ratios of p21/CUL1 were associated with <34 weeks of gestational age at delivery in PE. Further studies are needed to understand more clearly about the role of CUL1, VEGF, and p21 in PE.

Acknowledgments

The authors acknowledge Cipto Mangunkusumo Hospital dan Budi Kemuliaan Hospital for their provision of placental tissue, also all the women who donated placental tissues for this study. The authors acknowledge Integrated Laboratory Medical Faculty of Universitas Indonesia for giving its place to test the samples.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bdolah Y, Sukhatme VP, Karumanchi SA. Angiogenic imbalance in the pathophysiology of preeclampsia: Newer insights. Semin Nephrol 2004;24:548-56.
2. Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: The role of antiangiogenic factors and implications for later cardiovascular disease. Circulation 2011;123:2856-69.
3. Romero R, Chaiworapongsa T. Preeclampsia: A link between trophoblast dysregulation and an antiangiogenic state. J Clin Invest 2013;123:2775-7.
4. Reister F, Frank HG, Kingdom JC, Heyl W, Kaufmann P, Rath W, *et al*. Macrophage-induced apoptosis limits endovascular trophoblast invasion in the uterine wall of preeclamptic women. Lab Invest 2001;81:1143-52.
5. Woelkers D, Barton J, von Dadelszen P, Sibai B. [71-OR]: The revised 2013 ACOG definitions of hypertensive disorders of pregnancy significantly increase the diagnostic prevalence of preeclampsia. Pregnancy Hypertens 2015;5:38.
6. Liu W, Wang Y, Zhang C, Huang B, Bai J, Tian L. Cullin1 is up-regulated and associated with poor patients' survival in hepatocellular carcinoma. Int J Clin Exp Pathol 2015;8:4001-7.
7. Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, *et al*. Structure of the cul1–Rbx1–Skp1–F box Skp2 SCF ubiquitin ligase complex. Nature 2002;416:703-9.
8. Zhang Q, Chen Q, Lu X, Zhou Z, Zhang H, Lin H, *et al*. CUL1 promotes trophoblast cell invasion at the maternal–fetal interface. Cell Death Dis 2013;4:e502.
9. Chen J, Khalil RA. Matrix metalloproteinases in normal pregnancy and preeclampsia. Prog Mol Biol Transl Sci 2017;148:87-165.
10. Abbas T, Dutta A. p21 in cancer: Intricate networks and multiple activities. Nat Rev Cancer 2009;9:400-14.
11. Sharp AN, Heazell AE, Baczyk D, Dunk CE, Lacey HA, Jones CJ, *et al*. Preeclampsia is associated with alterations in the p53-pathway in villous trophoblast. PLoS One 2014;9:e87621.
12. Sultana Z, Maiti K, Dedman L, Smith R. Is there a role for placental senescence in the genesis of obstetric complications and fetal growth restriction? Am J Obstet Gynecol 2018;218:S762-73.
13. Masuyama H, Segawa T, Sumida Y, Masumoto A, Inoue S, Akahori Y, *et al*. Different profiles of circulating angiogenic factors and adipocytokines between early-and late-onset pre-eclampsia. BJOG 2010;117:314-20.
14. Moldenhauer JS, Stanek J, Warshak C, Khoury J, Sibai B. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. Am J Obstet Gynecol 2003;189:1173-7.
15. Gomathy E, Akurati L, Radhika K. Early-onset and late-onset preeclampsia-maternal and perinatal outcomes in a rural tertiary health center. Int J Reprod Contracept Obstet Gynecol 2018;7:2266-9.
16. Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: Updates in pathogenesis, definitions, and guidelines. Clin J Am Soc Nephrol 2016;11:1102-13.
17. Sgambati E, Marini M, Zappoli Thyrion GD, Parretti E, Mello G, Orlando C, *et al*. VEGF expression in the placenta from pregnancies complicated by hypertensive disorders. BJOG 2004;111:564-70.
18. Sezer SD, Küçük M, Döger FK, Yüksel H, Odabaşi AR, Türkmen MK, *et al*. VEGF, PIGF and HIF-1α in placentas of early- and late-onset pre-eclamptic patients. Gynecol Endocrinol 2013;29:797-800.
19. Li L, Wang M, Yu G, Chen P, Li H, Wei D, *et al*. Overactivated neddylation pathway as a therapeutic target in lung cancer. Natl Cancer Inst 2014;106:dju083.
20. Wang X, Li L, Liang Y, Li C, Zhao H, Ye D, *et al*. Targeting the neddylation pathway to suppress the growth of prostate cancer cells: Therapeutic implication for the men's cancer. Biomed Res Int 2014;2014:974309.
21. Samara TD, Liem IK, Prijanti AR, Andrijono. SEMA3B but not CUL1 as marker for pre-eclampsia progression. Malays J Med Sci 2019;26:66-72.

UNIVERSITAS TRISAKTI
FAKULTAS KEDOKTERAN
FACULTY OF MEDICINE - TRISAKTI UNIVERSITY
Jalan Kyai Tapa, Grogol, (Kampus B), Jakarta 11440, Indonesia
Telp. : (021) 5672731, 5655786 E-mail : fk@trisakti.ac.id
Faks : (021) 5660706 Web site : www.trisakti.ac.id/fk

S U R A T T U G A S

Nomor :4689.b/Usakti/FK/01.B/IX/2020

Dasar : Sehubungan dengan pengisian Rekam Jejak Beban Kerja Dosen (BKD) pada semester Ganjil/Genap Tahun Akademik 2020/2021 di Fakultas Kedokteran Universitas Trisakti.

Dekan Fakultas Kedokteran Universitas Trisakti

M E N U G A S K A N

Kepada : Dr. dr. Tjam Diana Samara,MKK

Untuk : Melaksanakan kegiatan Penelitian

Waktu : Semester Ganjil/Genap Tahun Akademik 2020/2021

Tempat : Fakultas Kedokteran Universitas Trisakti.

Demikian surat tugas ini untuk dilaksanakan dengan seksama dan penuh tanggung jawab.

Apabila dikemudian hari terdapat kekeliruan atau kesalahan dalam Surat Tugas ini, akan diubah dan diperbaiki sebagaimana mestinya.

Jakarta, 28 September 2020

D e k a n,

Dr. dr. Raditya Wratsangka, Sp.OG(K)
NIP : 196205271990031002/1588

KTU	WD1