SRIWIJAYA JOURNAL OF MEDICINE

# The Effect of EGFR Gene DNA Methylation on The Incident of Endometriosis

Anisah Nida'ul Haq[1], Ocktariyana[2*], Zen Hafy[3], Irsan Saleh[4]

[1]Master of Biomedical Sciences Program, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[2]Department of Midwifery, Ministry of Health Polytechnic, Palembang, Indonesia
[3]Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[4]Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
E-mail: ocktariyana@gmail.com

## Abstract

Endometriosis is a gynecological condition characterized by the development of endometrial tissue outside the uterus, often leading to pain and infertility. We explore the relationship between endometriosis and the effects of DNA methylation on the Epidermal Growth Factor Receptor (EGFR) gene. DNA methylation, an epigenetic mechanism, involves adding a methyl group to cytosine bases followed by guanine in CpG islands, thereby influencing gene expression through hypermethylation or hypomethylation. In endometriosis, methylation patterns on specific genes can lead to transcriptional changes, impacting inflammatory processes and hormonal functions, such as estrogen, that support the growth of ectopic tissue. Variations in the EGFR gene's DNA methylation are linked to elevated cellular activity and expression, which aids in the pathophysiology of endometriosis. These findings highlight the potential of DNA methylation as a therapeutic target in treating endometriosis, offering hope for improved patient outcomes.

**Keywords:** DNA methylation, epigenetics, endometriosis, EGFR

## 1. Introduction

Endometriosis is a benign yet progressive gynecological condition characterized by the ectopic growth of tissue that resembles the endometrium, which is the lining of the uterus. This tissue commonly develops outside the uterine cavity, particularly in the rectovaginal septum, the ovaries, and the pelvic peritoneum.[1] As this endometrial-like tissue undergoes cyclical changes in response to hormonal fluctuations, it can lead to significant complications, including chronic pelvic pain, dysmenorrhea, and potential fertility challenges.[2]

Endometriosis affects about 176 million women of reproductive age or 10% of the population.[3.4] According to a 2021 study by Santamera et al., the incidence of endometriosis is between 1.4 and 3.5 cases per thousand annually, while the prevalence is estimated to be between 1 and 5 percent.[5] Based on Dr. Mohammad Hoesin Hospital Palembang's medical record, 105 cases of endometriosis from 2018 to 2020 in women aged 15-49 years were diagnosed based on operative or laparoscopic procedures.[6]

Until now, the cause of endometriosis is still unknown; it is suspected that many factors influence it, such as hormonal, neurological, immunological, genetic, and epigenetic factors.[2] Recent studies have demonstrated that epigenetic abnormalities are one of the pathophysiologies of endometriosis.[7] Epigenetic regulation results in changes in chromatin conformation and DNA accessibility to its modulators, which can interfere with the transcription process of a gene.[8] Among the several epigenetic regulations that result in endometriosis is DNA methylation.[7]

The epigenetic process known as DNA methylation is defined by adding a methyl group to the cytosine base's 5' carbon chain on the CpG island.[8] DNA methylation can be in

the form of hypomethylation or hypermethylation of the promoter gene. Hypomethylation refers to a condition where there are decreased levels of methylation in the CpG islands located in the promoter region of a gene. This decrease typically leads to an increase in the expression of specific genes. In contrast, hypermethylation involves an increase in the methylation of these CpG islands in the gene promoter, which results in gene silencing and the inactivation of the transcription process.[9]

In endometriosis, it is suspected that the effect of methylation in the form of the addition of methyl groups has an impact on changes in gene expression, thereby affecting the functionality of endometrial cells.[10] The pathophysiology of endometrial disorders is believed to be influenced by DNA methylation in the endometrium, which dynamically occurs throughout the menstrual cycle.

## 2. Discussion

### 2.1. Methylation of Deoxyribonucleic Acid (DNA)

DNA methylation is believed to be involved in the progression of endometriosis.[8] In DNA methylation, a methyl group is attached to the fifth carbon of a cytosine base adjacent to a guanine in the 5'-CG-3' sequence. The methyl group originates from S-adenosyl-L-methionine (SAM), which is transferred to cytosine and catalyzed by the enzyme DNA methyltransferase (DNMT).[13] DNA methylation is inherited and can alter gene activity without changing the DNA sequence. CpG sites (methylation sites) are distributed 5-10% of the human genome, mainly in repetitive sequences outside CpG islands, and methylated by 70-80%. CpG island is a region containing many CpGs located around the promoter.

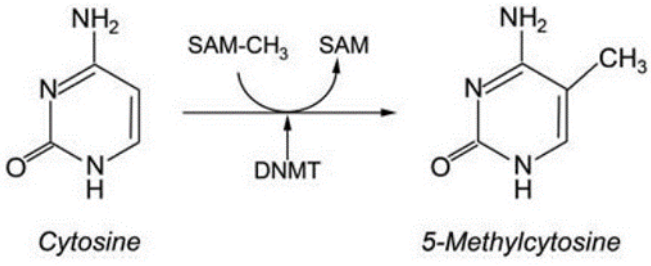


**Figure 1. Conversion of cytosine to 5-methylcytosine by DNA methyltransferase (DNMT).[14]**

In DNA methylation, two mechanisms affect gene expression: hypomethylation or hypermethylation. Hypomethylation refers to a decrease in CpG island methylation within the promoter region of a gene, resulting in increased gene expression. Conversely, hypermethylation occurs when CpG island methylation in the promoter of a gene increases, which can cause gene silencing and inactivation of the transcription process.[9]

### 2.2. DNA Methylation in Endometriosis

The DNA methyltransferase (DNMT) enzyme, which functions as a catalyst for the DNA methylation process, has been shown to have different expression patterns and functions at a relatively high level in the endometrial tissue of people living with endometriosis compared to the endometrium of healthy women.[9,12]

The development of endometriosis is closely associated with the estrogen pathway.

Two critical proteins control 17β-estradiol biosynthesis, namely steroidogenic (StAR) and aromatase (CYP19) expression. Both of these proteins have been shown to have increased expression in ectopic tissue and isolated stromal cells.

Another factor contributing to the development of endometriosis is increased PGE2 production brought on by elevated cyclooxygenase-2 (COX-2) expression. According to reports, ectopic stromal cells exhibit aberrant COX-2 induction due to hypomethylation of the COX-2 promoter, which increases the production of 17β-estradiol through positive feedback.[8]

Several genes that experience DNA methylation related to endometriosis in the CpG island promoter region are homeobox A10 (HOXA 10), splicing factor 1 (SF-1), estrogen receptor 2 (ESR-2), progesterone receptor (PGR), and Mitochondrially encoded Cytochrome c oxidase II (MT-CO2/COX2).[15-19]

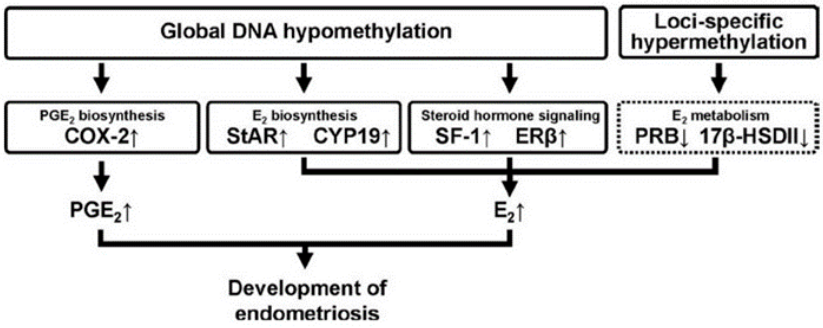


**Figure 2.DNA methylation enhances estradiol (E2) signaling and has a role in inflammation and steroidogenic capacity.[8]**

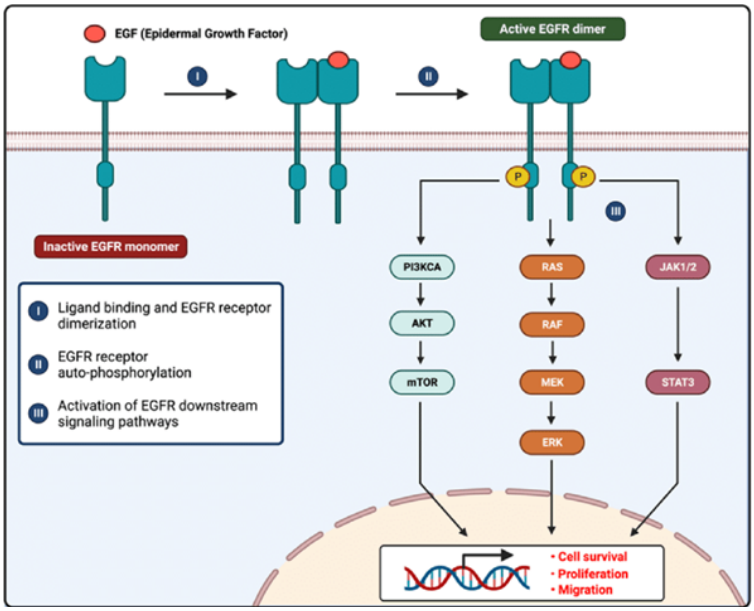


**Figure 3. Schematic of EGFR Activation and Signaling Pathway[21]**

### 2.3. Epidermal Growth Factor Receptor and Endometriosis

EGFR is a proto-oncogene from the ErbB/HER family of tyrosine kinase receptors, located on chromosome 7p12, encoded EGFR protein with a molecular weight of 170 kDa. EGFR plays a role in cellular phenotype transformation for tumor cell survival.[20]

The primary ligands that bind to the EGFR include epidermal growth factor (EGF), heparin-binding EGF, transforming growth factor-alpha (TGF-α), amphiregulin, betacellulin, and epiregulin. When EGFR is activated, it triggers several signal transduction pathways, specifically the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), phosphatidylinositol-3-kinase (PI3K)-Akt, signal transducers and activators of transcription (STATs), and phospholipase C gamma (PLC-γ). This pathway is a critical oncogenic regulator of tumor cell proliferation, invasion, angiogenesis, and metastasis.[22]

EGFR functions as a regulator of proliferation and differentiation in epidermal cells and generally has relatively low expression in normal endometrium.[23] Continuous activation or overexpression of the EGFR results in the aberrant activation of downstream signaling pathways. This condition contributes to increased cell proliferation, migration, malignant transformation, and tumorigenesis. Numerous studies have indicated that elevated levels of EGFR and its ligands serve as primary drivers of tumor growth and cancer progression.

Clinical studies have shown that in endometrial carcinoma, there is an increased expression of EGFR.[23] Increased EGFR expression has also been demonstrated in endometrial tumors.[24] Increased EGFR expression serves as a marker for tumor and cancer progression, indicating a poorer prognosis.[23] Some studies have reported that EGFR expression status is the most important prognostic factor for disease progression. Thus, increased expression of EGFR can be used to determine tumor and cancer prognostication, and inhibition of its cellular work predictively has therapeutic effects.

High or low expression is associated with the presence of hypomethylation or hypermethylation. A study by Montero et al. shows that EGFR hypermethylation is found in primary solid tumors in the breast, colorectal, lung, and head and neck, resulting in silencing or inactivating EGFR gene transcription.[20] Similar studies by Weng et al. showed that the EGFR gene is hypermethylated.[25] However, methylation is not the only mechanism that affects gene transcription; some genetic mechanisms and polymorphisms contribute to gene transcription. Previous studies indicate that genetic polymorphisms in intron-1 of EGFR, specifically the CA simple sequence repeat (SSR), influence the transcription of the EGFR gene.[20] Among the various somatic mutations of EGFR in NSCLC, exon 19 deletions and mutations in the EGFR domain are the most common, accounting for 85% of all EGFR mutations.[26]

## 3. Conclusion

By knowing the epigenetic mechanism, especially DNA methylation related to the pathogenesis of endometriosis, in the future, it will be possible for DNA methylation analysis to be used as a tool for early detection, diagnosis, and treatment. The ongoing research in DNA methylation detection can help develop epigenetic biomarkers.

## References

1. Zondervan KT, Becker CM, Missmer SA. Endometriosis. Longo DL, editor. New England Journal of Medicine [Internet]. 2020 Mar 26;382(13):1244–56.

2. Saunders PTK, Horne AW. Endometriosis: Etiology, pathobiology, and therapeutic prospects. Vol. 184, Cell. Elsevier BV; 2021. p. 2807–24.
3. Marinho MCP, Magalhaes TF, Fernandes LFC, Augusto KL, Brilhante AVM, Bezerra LRPS. Quality of Life in Women with Endometriosis: An Integrative Review. Vol. 27, Journal of Women's Health. Mary Ann Liebert Inc.; 2018. p. 399–408.
4. Eisenberg VH, Weil C, Chodick G, Shalev V. Epidemiology of endometriosis: a large population-based database study from a healthcare provider with 2 million members. BJOG: An International Journal of Obstetrics and Gynecology. Blackwell Publishing Ltd; 2017
5. Sarria-Santamera A, Orazumbekova B, Terzic M, Issanov A, Chaowen C, Asúnsolo-Del-barco A. Systematic review and meta-analysis of incidence and prevalence of endometriosis. Vol. 9, Healthcare (Switzerland). MDPI AG; 2021.
6. Leriva RT, Iskandar Zulqarnain, Hadrians Kesuma Putra, Awan Nurtjahyo, Syifa Alkaf. Characteristics of Endometriosis Patients in Dr. Mohammad Hoesin General Hospital from 2018 to 2020. Bioscientia Medicina: Journal of Biomedicine and Translational Research. 2022 Feb 4;6(4):1592–7.
7. Ocktariyana O. Analysis of mRNA expression and DNA methylation level of RAC1 gene encoding focal adhesion molecule in endometrial end peritoneal endometriosis. 2020.
8. Hsiao KY, Wu MH, Tsai SJ. Epigenetic regulation of the pathological process in endometriosis. Vol. 16, Reproductive Medicine and Biology. John Wiley and Sons Ltd; 2017. p. 314–9.
9. Grimstad, F.W. and Decherney, A. (2017) 'A review of the epigenetic contributions to endometriosis', Clinical Obstetrics & Gynecology, 60(3), pp. 467–476.
10. Xue Q, Lin Z, Cheng YH, Huang CC, Marsh E, Yin P, et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. Biol Reprod. 2007 Oct;77(4):681–7.
11. Kukushkina V, Modhukur V, Suhorutšenko M, Peters M, Mägi R, Rahmioglu N, et al. DNA methylation changes in endometrium and correlation with gene expression during the transition from pre-receptive to receptive phase. Sci Rep. 2017 Dec 1;7(1).
12. Koukoura O, Sifakis S, Spandidos DA. DNA methylation in endometriosis (Review). Vol. 13, Molecular Medicine Reports. Spandidos Publications; 2016. p. 2939–48
13. Kokcu A. A current view of the role of epigenetic changes in the etiopathogenesis of endometriosis. Vol. 36, Journal of Obstetrics and Gynecology. Taylor and Francis Ltd; 2016. p. 153–9
14. Gibney ER, Nolan CM. Epigenetics and gene expression. Vol. 105, Heredity. 2010. p. 4–13.
15. Andersson KL, Bussani C, Fambrini M, Polverino V, Taddei GL, Gemzell-Danielsson K, et al. DNA methylation of HOXA10 in eutopic and ectopic endometrium. Human Reproduction. 2014;29(9):1906–11.
16. Xue Q, Lin Z, Yin P, Milad MP, Cheng YH, Confino E, et al. Transcriptional activation of steroidogenic factor-1 by hypomethylation of the 5' CpG island in endometriosis. Journal of Clinical Endocrinology and Metabolism.

2007;92(8):3261–7.
17. Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor isoform B (PR-B) in endometriosis. Epigenetics. 2006;1(2):106–11.
18. Zidan HE, Rezk NA, Alnemr AAA, Abd el Ghany AM. COX-2 gene promoter DNA methylation status in eutopic and ectopic endometrium of Egyptian women with endometriosis. J Reprod Immunol. 2015 Nov 1;112:63–7.
19. Darmawi. Degree of DNA Methylation and mRNA Expression Level of Progesterone Receptor B (PR-B) Gene in Endometriosis Peritoneum Tissue, Endometrioma, Endometrium and Menstrual Blood of Endometriosis Patients. 2018.
20. Indonesian Reproductive Endocrinology and Fertility Association (HIFERI) Indonesian Obstetrics and Gynecology Association (POGI). Consensus on the management of endometriosis pain First Revision 2017.
21. Harrison DA. The JAK/STAT pathway. Cold Spring Harb Perspect Biol. 2012 Mar;4(3).
22. Kalyankrishna S, Grandis JR. Epidermal growth factor receptor biology in head and neck cancer. Vol. 24, Journal of Clinical Oncology. 2006. p. 2666–72.
23. Xu Y, Tong J, Ai Z, Wang J, Teng Y. Epidermal growth factor receptor signaling pathway involved in progestin-resistance of human endometrial carcinoma: In a mouse model. Journal of Obstetrics and Gynecology Research. 2012 Dec;38(12):1358–66.
24. Konecny GE, Santos L, Winterhoff B, Hatmal M, Keeney GL, Mariani A, et al. HER2 gene amplification and EGFR expression in a large cohort of surgically staged patients with non-endometrioid (type II) endometrial cancer. Br J Cancer. 2009 Jan 13;100(1):89–9
25. Weng X, Zhang H, Ye J, Kan M, Liu F, Wang T, et al. Hypermethylated epidermal growth factor receptor (EGFR) promoter is associated with gastric cancer. Sci Rep. 2015 May 11;5.
26. Chong CR, Jänne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. Vol. 19, Nature Medicine. 2013. p. 1389–40