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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Epigenetic regulation through estrogen and its receptors in inflammatory bowel disease model

研究課題名（英文）Epigenetic regulation through estrogen and its receptors in inflammatory bowel disease model

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：今回の研究では、エストロゲンがその受容体であるERβとGPR30を介して大腸炎に対して保護的に作用していることが示唆された。エストロゲンのシグナル伝達は、ゲノムと非ゲノムの両方の経路へ作用している。さらにエピジェネティックの調節因子であるHDACのSAHAは、炎症を誘発するサイトカインやケモカインの局所的な分泌を低下させ、炎症細胞の蓄積や動員を抑制することで、DSSにより誘発された大腸の炎症性変化を軽減していた。詳細なエピジェネティック解析により、H3K36の発現が大腸の炎症と関連していた。

研究成果の学術的意義や社会的意義
Estrogen targeted epigenetic therapy will help to develop new treatment approach for inflammatory bowel disease. Our results clearly indicated that estrogen has protective effect through its receptor GPR30 in mouse colitis model.

研究成果の概要（英文）：The results of this study suggest that estrogen has protective effect in colitis through its receptor ERβ and GPR30. Estrogen signaling acts through both genomic and non-genomic pathways. Moreover, epigenetic regulator HDAC, suberoylanilide hydroxamic acid attenuates inflammatory changes in DSS-induced colitis by suppressing local secretion of pro-inflammatory cytokines and chemokines and also by suppressing mobilization and accumulation of inflammatory cells. Detailed epigenetic analysis was detected that H3K36 expression was associated with colonic inflammation.

研究分野：解剖学

キーワード：estrogen　epigenetics　colitis

１．研究開始当初の背景

Multi-centered, population based epidemiologic studies consistently demonstrated greater incidence of inflammatory bowel diseases (IBD) in female than males, which shows possible involvement of estrogen signaling in the colitis pathogenesis (Bernstein et al. Am J Epidemiol. 149:916–924). In IBD pathogenesis, the epigenetics is known as an important regulating factor. The epigenetics is the functionally relevant modifications to the genome, without altering the DNA sequence, but having changes in DNA methylation and histone modification. Recently, the epigenetic therapy have shown effective in inflammatory models including rheumatoid arthritis, multiple sclerosis and psoriasis, however, the epigenetic treatment effect in IBD is largely unknown (Fig. 1).

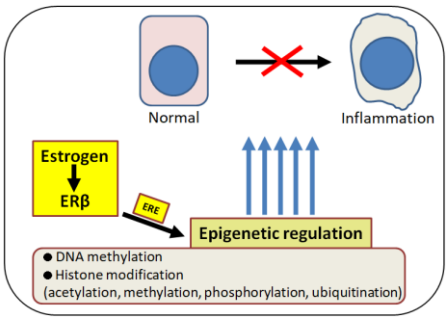

Fig.1. Proposed estrogen signaling in inflammatory bowel disease

We first described the expression of functional ERβ in mouse intestine by immunohistochemistry (Fig. 2) (Choijookhuu et al. Clin Res Hepatol Gastroenterol. 39, 499-507, 2015; Choijookhuu et al. Histochem Cell Biol 137:575-587, 2012). While ERβ is involved in protection of intestinal epithelium, the estrogen targeted therapy could be useful for IBD treatment.

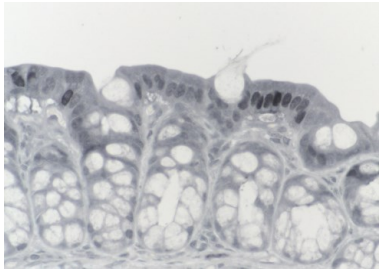

Fig.2. Immunolocalization of ERß expression in mouse colon.

２．研究の目的

Our purpose is to investigate the role of estrogen and its receptor through epigenetic regulation in IBD. It is already known that women are more affected than men by IBD incidence, which giving us the possibility to develop hormonal treatment approach in clinical gastroenterology. Discovering the molecular mechanisms of epigenetic regulation of IBD will become fundamental research for epigenetic therapy for IBD in the worldwide.

３．研究の方法

C57BL/6J wildtype mouse were used in this research. 1.5% DSS will be given with drinking water for 5 consequent days. To study the effect of estrogen in epigenetic regulation, 17ß-estradiol was treated. Disease activity index was evaluated during DSS treatment and histological scoring was performed in mouse colon tissue. The localization of GPR30 and HIF-1a, H3K36 was determined by immunohistochemistry.

４．研究成果

1. Histopathological evaluation was performed in DSS and E_2-treated mouse colon. In DSS-treated mouse colon, acute inflammation was observed, including shortening and loss of crypts and infiltration of inflammatory cells in the lamina propria. However, these changes were

significantly decreased in DSS-E_2-treated mouse colon. Macrophotography provides shortening of colon length in DSS-treated mouse, however it was preserved in DSS+E_2 mouse (Fig. 3).

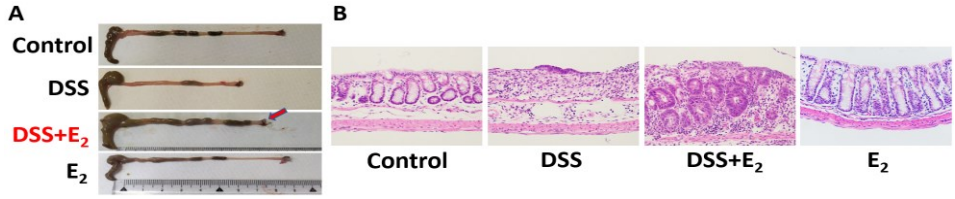

Fig.3. Colon length (a) and HE staining (b) in DSS and E_2-treated mice.

2. We used immunohistochemistry to determine the expression of GPR30 in the colon. The increased expression of GPR30 was found in E_2 treated mouse colon. However, GPR30 was decreased in DSS treated colon, especially in inflamed areas. Protective effect of E_2 may acting via GPR30 in DSS+E_2-treated mice (Fig. 4). These results suggest that protective effect of estrogen acts through GPR30, which transducted by non-genomic pathway.

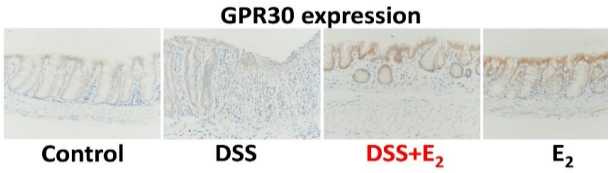

Fig.4. GPR30 expression in DSS and E_2-treated mice.

3. We investigated the effects of the epigenetic regulator, histone deacetylase (HDAC) inhibitor, suberoylanilide hydroxamic acid (SAHA), in a mouse model of DSS-induced colitis. In DSS treated mouse colon, the abscess and infiltration of inflammatory cells into the lamina propria and submucosa, was found in DSS-treated mouse colon. Surprisingly, DSS+SAHA-treated mouse colon revealed only mild damage on all days compared to DSS-treated mouse colon. In RT-PCR results, the highest expression levels of IL-6 and TNF-α were found in DSS-treated mouse colon on days 5 and 12, whereas significantly lower expression was found in DSS+SAHA-treated mouse (Fig. 5).

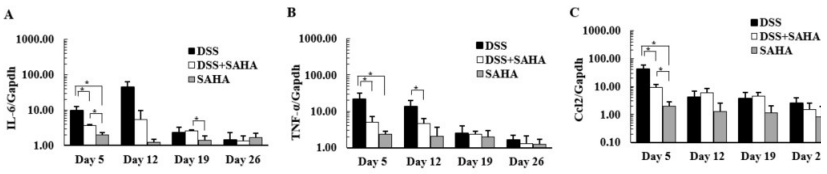

Fig.5. IL-6, TNF-α, Ccl2 in DSS and SAHA treated mouse colon.

4. To determine epigenetic regulation we examined by methylated histone proteins in DSS and E2 treated mouse colon. After thorough examination of H3K9, H3K14, H3K18 and H3K27 in mouse colon samples, we found that H3K36 was related to colonic inflammation, that significantly decreased in DSS treated mouse (Fig. 6).

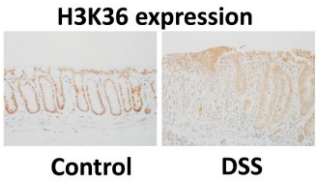

Fig.6. H3K36 expression in DSS and E_2-treated mouse.

In summary, all these results suggest that estrogen has protective role in colitis, through its receptors GPR30. Moreover, epigenetic regulator HDAC, attenuates inflammatory changes in DSS-induced colitis by suppressing local secretion of pro-inflammatory cytokines and chemokines and also by suppressing mobilization and accumulation of inflammatory cells.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　1件）

１．著者名 Srisowanna N, Choijookhuu N, Yano K, Batmunkh B, Ikenoue M, Mai NNH, Yamaguchi Y, Hishikawa Y	４．巻 52
２．論文標題 The effect of estrogen on hepatic fat accumulation during early phase of liver regeneration after partial hepatectomy in rats	５．発行年 2019年
３．雑誌名 Acta Histochemica et Cytochemica	６．最初と最後の頁 67-75
掲載論文のDOI（デジタルオブジェクト識別子） 10.1267/ahc.19018	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Palasin K, Uechi T, Yoshihama M, Srisowanna N, Choijookhuu N, Hishikawa Y, Kenmochi N, Chotigeat W	４．巻 9
２．論文標題 Abnormal development of zebrafish after knockout and knockdown of ribosomal protein L10a	５．発行年 2019年
３．雑誌名 Scientific Reports	６．最初と最後の頁 1-11
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-019-54544-w	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Diep NV, Choijookhuu N, Ozawa M, Fuke N, Hishikawa Y, Yamaguchi R	４．巻 -
２．論文標題 New tropisms of porcine epidemic diarrhoea virus (PEDV) in pigs naturally coinfected by variants bearing large deletions in the spike (S) protein and PEDVs possessing an intact S protein	５．発行年 2020年
３．雑誌名 Transboundary and Emerging Diseases	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/tbed.13607	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Fukui T, Fukaya T, Uto T, Takagi H, Nasu J, Miyanaga N, Nishikawa Y, Koseki H, Choijookhuu N, Hishikawa Y, Yamashita Y, Sato K	４．巻 -
２．論文標題 Pivotal role of CD103 in the development of psoriasiform dermatitis	５．発行年 2020年
３．雑誌名 Scientific Reports	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Choijookhuu N, 石塚 匠, 徐 岩, 小路 武彦, 菱川 善隆
２．発表標題 A new approach for in situ hybridization using fluorescence resonance energy transfer based molecular beacon probe
３．学会等名 第60回日本組織細胞化学, 第13回日中（国際学会）
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

宮崎大学医学部解剖学講座組織細胞化学分野 http://www.med.miyazaki-u.ac.jp/home/anatomy1/

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関