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学位授与の要件	学位規則第４条第１項該当
学位論文題目	12-O-tetradecanoylphorbol-13-acetate induces Epstein-Barr virus reactivation via NF-κB and AP-1 as regulated by protein kinase C and mitogen-activated protein kinase （TPA はプロテインキナーゼ C と MAP キナーゼにより調節された NF-κB と AP-1 を介して EB ウイルス再活性化を誘導する）
学位論文審査委員	（主査）佐藤建三 （副査）箸本英吉　　西連寺剛

学位論文の内容の要旨

EBV is a human herpes virus that infects B cells and epithelial cells to establish a latent infection. It is associated with many malignant diseases such as Burkitt's lymphoma, nasopharyngeal carcinoma and gastric carcinoma. TPA is a protein kinase C activator and tumor promoter, it can induce EBV reactivation. However, the molecular mechanism of EBV reactivation remains unknown. Previously the authors found that nitric oxide (NO) inhibited EBV reactivation and TPA inhibited iNOS expression in EBV infected gastric epithelial cell line GT38. This study has characterized the intracellular signaling pathways by which TPA induces EBV reactivation in GT38 cells. Spontaneous EBV reactivation became undetectable upon long-term culturing of GT38 cells, while iNOS mRNA expression as a marker of NO production increased. The PKC inhibitors H7 and staurosporine and MAPK inhibitor PD98059 inhibited TPA-induced expression of EBV immediate early genes BZLF1 and BRLF1 and reversed TPA-mediated inhibition of iNOS gene expression. NF-κB and AP-1 were also activated by TPA in a time-dependent manner. The TPA-induced NF-κB activation and BZLF1 expression were inhibited by pretreatment with NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC). These results demonstrats that TPA induces EBV reactivation via NF-κB and AP-1 and that PKC is an important mediator in regulating gene expression leading to EBV reactivation after TPA treatment of GT38 cells.

方　法

GT38 cells were grown in RPMI 1640 medium, supplemented with 10% fetal bovine serum.

Total RNA from cells was prepared by an ISOGEN kit. BZLF1, c-*jun* or *jun*B mRNA were detected by Northern blot analysis. iNOS mRNA expression was analysed by RT-PCR Southern blotting. ZEBRA was detected by Western blot analysis using anti-ZEBRA antibody. Nuclear transcription factors NF-kB and AP-1 were analyzed by electrophoretic mobility shift assays.

結　果

The expression of ZEBRA was highest in GT38-96 cells , and it was much higher in GT38-97 cells than in GT38-98 cells. In contrast to ZEBRA, iNOS mRNA expression was highest in GT38-98 cells, and it was much higher in GT38-97 cells than in GT38-96 cells. TPA-induced expressions of BZLF1 and BRLF1 were greatly inhibited by pretreatment with H7 , staurosporine or PD98059. On the other hand, TPA markedly inhibited iNOS mRNA expression and that inhibition could be reversed by pretreatment with H7, staurosporine, and PD98059. NF-κB binding activity rapidly increased and peaked at 0.5 h after TPA treatment. TPA treatment further increased the level of activated AP-1 in the nuclear extracts. NF-κB inhibitor, PDTC inhibits NF-κB activity and BZLF1 expression. c-*Jun* was increased significantly at 0.5 h, reached a peak at 2 h, and declined to a low level at 24 h after TPA stimulation. *jun*B was increased slightly at 0.5 h, reached a plateau at 1 h, and maintained a high level at least until 24 h after TPA treatment.

考　察

This study investigated the molecular mechanisms by which TPA inhibits iNOS gene expression and induces EBV reactivation in the gastric tissue derived epithelial cell line GT38 cells firstly. This paper demonstrated that the expression of ZEBRA was highest in GT38-96 cells. In contrast to ZEBRA, iNOS mRNA expression was highest in GT38-98 cells. Secondly the paper demonstrated that the involvement of PKC pathway in TPA-induced EBV reactivation. This observation has led to a better insight into the signal transduction pathway associated with EBV reactivation. The results indicate that TPA can induce AP-1 binding activity, suggesting that BZLF1 expression might be regulated by AP-1. NF-κB is also involved in the up-regulation of BZLF1 by TPA treatment, and the BZLF1 promoter domain might also contain binding sites for the transcription factor NF-κB. Regulation of the BZLF1 and BRLF1 promoters in GT38 cells may be mediated, in part, through a homodimer of c-Jun or c-Jun/JunB heterodimer.

結　論

This study, for the first time, demonstrates that TPA-induced EBV reactivation can occur by activating NF-κB and AP-1, and suggests that this reactivation, marked by BZLF1 expression, is likely to be mediated at least via both PKC and MAPK pathways in the gastric tissue-derived epithelial cell line GT38.

論 文 審 査 の 結 果 の 要 旨

本研究はEBウイルス（EBV）が感染してる胃上皮細胞株GT３８においてTPAによるEBV再活性化のシグナル伝達機構を解析したものである。筆者らは先に一酸化窒素（NO）が細胞内に潜伏するEBV再活性化を抑制することを見出した。本研究において、EBV再活性化誘導物質として良く知られているTPAは、PKCを活性化しiNOS遺伝子の発現を抑制し、NO合成を阻害する結果EBV再活性化を誘導すること、さらにTPAはPKCおよびMAPKを活性化し、その下流の転写因子AP-1とNF-κBの活性化を介してEBV再活性化を誘導することを明らかにした。

本研究は、TPAが細胞内に潜伏するEBVを再活性化する機構として、PKC活性化からのシグナル伝達経路を明らかにしたものであり、EBVの分子生物学分野において明らかに学術水準を高めたものと認める。