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論文題目	Physiological concentrations of glucocorticoids induce pathological DNA double-strand breaks （生理濃度の糖質コルチコイドは病的なDNA二重鎖切断を引き起こす）		

（論文内容の要旨）

Nuclear receptors induce rapid transcriptional responses as transcription factors upon binding to ligands, including androgen, estrogens, and corticosteroids together termed 'steroid' hormones. Corticosteroids include two main classes, glucocorticoids (GCs) and mineralocorticoids. Cortisol is a crucial GC in the human body and is essential for life. GCs bind to the glucocorticoid receptor (GR). Ligand-bound GR regulates the transcription of numerous GR-target genes as a transcription factor. GR-mediated signaling suppresses immune cells. Dexamethasone (Dex), a synthetic GC, has been widely used clinically for suppressing immune reactions in patients with allergies, autoimmune diseases, organ transplantations, and COVID-19. Cortisol binds to both GR and mineralocorticoid receptors, whereas Dex binds only to GR. Dex has ~50 times more potent GR-stimulating activity than cortisol. Serum cortisol is at 80 – 700 nM, and the clinically relevant concentration of Dex is 6 – 250 nM.

DNA Topoisomerase II (TOP2) plays a vital role in transcriptional responses to various extracellular signals, including nuclear-receptor ligands, cytokines, neurotransmitters, heat shock, and transcriptional elongation. TOP2 catalyzes topological changes by strand passage reactions, where one intact double-stranded DNA duplex passes through a transiently formed enzyme-bridged break in the other DNA (gated helix). The enzyme-bridged break consists of a TOP2 homodimer covalently bound to the 5' DNA ends of the break, forming TOP2-DNA cleavage-complex intermediates (TOP2ccs). TOP2 seals TOP2ccs and is released from rejoined genomic DNA. TOP2 often fails to seal, called 'abortive catalysis' leading to the generation of cytotoxic DSBs called stalled TOP2ccs. Re-ligation of stalled TOP2ccs is carried out by coordinated reactions of Tyrosyl-DNA phosphodiesterase 2 (TDP2) and non-homologous end-joining (NHEJ). NHEJ plays the dominant role in DSB repair during G0/G1 phases and requires DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and Ligase IV. NHEJ is incapable of joining DSB ends carrying blocking adducts such as 5' TOP2 adducts. TDP2 removes 5' TOP2 adducts from stalled TOP2ccs before ligation by NHEJ. TDP2 and BRCA1-MRE11 are complementary to each other in removing 5' TOP2 adducts.

A sex hormone, dihydrotestosterone, causes persistent TOP2-dependent DSBs at its physiological concentrations. Likewise, physiological concentrations of estrogens are highly genotoxic, and this genotoxicity is dependent on both estrogen receptors (ERs) and TOP2. These data demonstrated the frequent formation of stalled TOP2ccs during the early transcriptional response to the sex hormone. However, GCs are believed to have no detectable genotoxicity or carcinogenicity since they are one of the most widely prescribed drugs. Previous studies poorly studied GC-induced DSBs at serum concentrations.

This paper revealed that the exposure of primary lymphoid cells to Dex at a clinically relevant concentration (10 nM) induced one γH2AX focus, the biomarker of individual DSBs, per cell on average, even in the presence of proficient DSB repair. A physiological concentration (100 nM) of cortisol and 10 nM of Dex induced multiple γH2AX foci in each G_1 phase cell deficient in BRCA1, TDP2, or NHEJ. This article demonstrated that cortisol and Dex induce DSBs by activating GR and generating stalled TOP2ccs in G_0/G_1 cells. NHEJ very efficiently seals these DSBs as DSB-repair-proficient cells poorly exhibited GC-induced γH2AX and 53BP1 foci. This conclusion agrees with the fact that GCs do not have any mutagenic potential. The inhibition of RNA polymerase II reduced the number of cortisol-induced DSBs only less than 50% in the G_1 phase, which data agree with estrogen-induced DSBs. The data suggested the frequent formation of irreversible TOP2ccs at transcriptional regulatory sequences during the early transcriptional response since it involves active TOP2 catalysis at enhancers and promoters. In conclusion, cortisol and Dex generate many TOP2-dependent DSBs during transcriptional induction of target genes.

（論文審査の結果の要旨）

副腎皮質ホルモンの一つであるコルチゾールは、主要な糖質コルチコイドである。化学合成された糖質コルチコイドであるデキサメタゾンは、炎症を抑制するために臨床で広く用いられる。本研究は、生理濃度のコルチゾールや、臨床で使用される濃度のデキサメタゾンが持つ、潜在的な DNA 毒性を明らかにした。

副腎皮質ホルモンは、転写を誘導する。その際にトポイソメラーゼ II (TOP2) が働く。TOP2 は、DNA の絡まりを取り除く酵素である。TOP2 は、触媒反応中に DNA と共有結合して、一過的に DNA の二本鎖切断 (DSB) を引き起こす。TOP2 は、DNA の再結合にしばしば失敗する。TOP2 の触媒不全で生じた DSB は、TDP2 ホスホジエステラーゼと非相同末端結合（NHEJ）により修復される。

本論文は、TDP2 や NHEJ が働かないヒト培養細胞とマウスにおいて、コルチゾールやデキサメタゾンが、G1 期に効率的に DSB を発生させることを明らかにした。DSB 形成は、糖質コルチコイド受容体と TOP2 に依存した。TDP2 は、DSB 末端から TOP2 を除去する特異的な酵素である。そのため、上記で観察された DSB は、TOP2 の触媒停止が原因である可能性が最も高いと結論づけた。本研究は、コルチゾールやデキサメタゾンが頻繁に DSB を生成することを初めて明らかにした点で、当該分野に貢献した。その成果は、論文提出者に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和５年１月１７日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降