ATM suppresses c-Myc overexpression in the mammary epithelium in response to estrogen(Abstract_要旨)

AUTHOR(S):

Najnin, Rifat Ara

京都大学	博士（　医　学）	氏　名	Rifat Ara Najnin
論文題目	ATM suppresses c-Myc overexpression in the mammary epithelium in response to estrogen （ATM は乳腺上皮細胞においてエストロゲンに応答した c-Myc の過剰発現を抑制する）		

（論文内容の要旨）

DNA double-strand breaks (DSBs) can result in highly carcinogenic mutations such as chromosome translocation and long deletion in the genomic DNA. DSBs are generated not only by environmental factors such as γ-rays and etoposide (anti-cancer topoisomerase II (Top2) poison) but also by endogenous causes such as 'abortive' catalysis by Top2. Abortive catalysis generates DSBs called the DNA-Top2 complex (TOP2ccs), where Top2 covalently binds to DSB ends. Top2 controls the topology of DNA by transiently introducing DSBs and plays essential roles in DNA replication and transcription. Estrogens activate transcription of their target genes, and this activation is associated with very active catalysis of Top2ß, one of the two Top2s along with Top2α.

ATM controls the cellular response to DSBs. In response to DSBs, the ATM kinase is immediately activated to stabilize the p53 tumor suppressor protein via phosphorylation and stimulate the DNA damage checkpoint pathway. ATM promotes homology-directed repair (HDR), which functions as the dominant DSB repair pathway during DNA replication. Although the damage checkpoint and HDR prevent mutagenesis in all cycling cells, the mechanism by which the phenotype resulting from defective ATM is manifested selectively in estrogen-regulated tissue remains a central unresolved question in ATM biology.

The effect of these DSBs at enhancers on the transcriptional response when stalled TOP2ccs are left unrepaired is also unknown as investigations of this issue are hindered by difficulties in measuring the activation kinetics of enhancers during the response to extracellular signals. While genome-wide analyses of epigenetic markers have uncovered many constitutively active enhancers, the ability to identify enhancers that activate target genes only transiently in response to extracellular stimuli remains a challenge. Nevertheless, the activity of extracellular stimuli-dependent enhancers can be measured by identifying enhancer RNAs (eRNAs), which are bi-directionally transcribed from active enhancers, as their activity correlates with eRNA expression. eRNAs can be detected using an approach known as Cap Analysis of Gene Expression (CAGE), in which RNAs are sequenced from the 5' RNA cap. However, its sensitivity is limited by the short average half-life of eRNAs (only 1 min), which is approximately 50 times shorter than that of mRNAs. As a new CAGE method, Native Elongating Transcript-CAGE (NET-CAGE) overcomes this problem by selectively examining nascent RNAs complexed with RNA polymerase II (Pol2) and undergoing productive elongation *in vivo*. To prevent the release of paused Pol2 during *in vitro* RNA extraction, NET-CAGE involves the extraction of RNA with the Pol2 inhibitor, α-amanitin. These methods allow NET-CAGE to accurately measure the activation kinetics of both individual enhancers and their target promoters in response to extracellular signals.

In the current study, NET-CAGE revealed that the defective repair of stalled TOP2ccs in cells deficient in ATM and TDP2 significantly changed the activation kinetics of enhancers in response to E2. The data led to hypothesize that stalled TOP2ccs occur frequently at estrogen-dependent enhancers of oncogenes and defective DSB repair promotes oncogenesis by causing dysregulation of the response of oncogenes to estrogen.

This study aimed to better understand the mechanism by which the risk of oncogenesis is dramatically enhanced upon selective LOH of the *ATM* gene in mammary epithelial cells. To this end, I explored the potential role of ATM in estrogen-mediated oncogenesis in mammary tissue. In brief, I revealed that ATM is required for repairing stalled TOP2ccs, which occur at enhancers of the *c-MYC* gene in human BC cells during estrogen exposure. Defective repair of such DSBs causes overexpression of the c-Myc protein in human BC cells following E2 exposure and in mammary epithelial cells upon an intraperitoneal (i.p.) injection of E2 into mice, with the high expression continuing for 24 h. Our study highlights the role of ATM in suppressing the strong proliferative effect of estrogens and preventing ER+ BC development.

（論文審査の結果の要旨）

ATM 遺伝子の変異保因者は、エストロゲン受容体陽性の乳癌になりやすい。ATM は、p53 遺伝子を活性化して乳癌の発癌を防ぐ。しかし、*ATM* 欠損後の乳腺組織特異的な発癌機構は未解明である。本研究は、エストロゲン暴露後の早期転写応答を ATM が制御することを明らかにした。エストロゲン暴露後の転写応答は、トポイソメラーゼ II（TOP2）に依存する。TOP2 は、DNA の二本鎖切断（DSB）と再結合を繰り返す酵素である。本研究では、TOP2 が自ら切断した DNA を再結合できない場合に、ATM がその修復を促進することを見出した。エストロゲンに暴露したヒト乳癌細胞では、TOP2 による DSB が *c-MYC* 癌遺伝子の転写調節領域（エンハンサー）に生じた。DSB が修復されないとエンハンサーの活性化状態が変化し、*c-MYC* を含む多くの遺伝子が過剰発現した。エンハンサーを人為的に切断すると *c-MYC* が過剰発現したことから、エンハンサーの DSB が *c-MYC* の過剰発現を誘導すると示唆された。エストロゲン投与により、*Atm* 欠損マウスの乳腺上皮細胞で、c-Myc タンパク質の過剰発現と c-Myc 依存的な細胞の過剰増殖がみられた。以上の研究は、エストロゲンによる c-Myc 依存的な強い細胞増殖作用を ATM が抑制することを明らかにした点で、ATM の組織特異的発癌の解明に貢献する。

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４年２月１５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