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TLS-CHOP キメラ遺伝子に制御される lncRNA の解析

（大学院修士課程 2 年分子病理学）
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粘液型脂肪肉腫は、脂肪肉腫全体の 15-20%、成人の軟部肉腫全体の 5% を占める、比較的頻度の高い肉腫である。その発生原因は、転座により 16p11 に位置する TLS 遺伝子と 12q13 に位置する CHOP 遺伝子が融合することにより生じるキメラ遺伝子（TLS-CHOP）と考えられている。しかしながら、TLS-CHOP 下流の分子機構は依然として不明な点が多い。

一方、ヒトゲノムのおよそ 98% の領域からタンパク質をコードしないノンコーディング RNA（ncRNA）が大量に転写されていることが明らかとなっている。ncRNA には、比較的機能解析が進んでいる tRNA、rRNA、miRNA など以外に、依然として機能が不明な点が多い長鎖 ncRNA（lncRNA）がある。lncRNA の一部は、転写や翻訳、エピジェネティクスの制御などを介して、細胞分化、個体発生、がんを含む各種疾患の発症など、生体の多様なプロセスに関与することが明らかになりつつあるが、大部分の lncRNA の役割は不明である。

以上のことから、本研究は、TLS-CHOP に制御される lncRNA の同定と機能解析から粘液型脂肪肉腫の発症機構の解明を目的とした。

近年、各種肉腫の Tumor initiating cells（TICs）（がん幹細胞）は、間葉系幹細胞由来であることを示す報告が相次いで発表されている。そこで、間葉系幹細胞に TLS-CHOP を導入し、粘液型脂肪肉腫の発がんモデルの構築を試みた。間葉系幹細胞にレンチウイルスを用いて TLS-CHOP キメラ遺伝子を導入し、マイクロアレイを用いて TLS-CHOP が発現制御する遺伝子の網羅的な解析を行った。その結果から、TLS-CHOP を導入した 2 種類の間葉系幹細胞で共通して発現が変動する遺伝子を抽出し、その中から TLS-CHOP によって発現が誘導される 1 つの lncRNA および発現が抑制される多数の lncRNA を同定した。また、qPCR を用いてそれら lncRNA の発現変動の再検証を行った。続いて、TLS-CHOP によって発現が変動する lncRNA の機能解析から、粘液型脂肪肉腫の発症機構への関与を検証する。

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A high titer anti-factor XI autoantibodies which were identified from the patient with complicated laboratory results

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【Background】 Acquired coagulation inhibitors developed in patients with previously normal haemostasis can cause life-threatening bleeding. Its diagnosis may be difficult in routine clinical laboratory settings. We tried to clarify the pathological condition of a patient who expressed recurrent spontaneous bleeding in her age 40s with an obvious APTT prolongation and complicated laboratory results.
【Methods/Results】 Cross-mixing test showed lupus anticoagulant（LA）pattern. However, Gradipore-LA（dRVVT test）and Staclot LA（APTT-LA）assay were negative. Anti-phospholipids antidodies（anti-cardiolipin antibody, anti-β2-glycoprotein I antibody, and phosphatidylserine-dependent anti-prothrombin antibody）were also negative. All intrinsic coagulation factors（factors VIII, IX, XI, and XII）, measured by usual one-stage clotting assays, showed a significant reduction. The presence of high titer antibodies to the intrinsic coagulation factors was detected by Bethesda assay. On the other hand, activity measurements of coagulation factors with multi-diluted samples suggested a presence of antibody against factor XI（FXI）in the patient's plasma. In order to elucidate the character of the antibody we purified IgG by using protein A/G from plasma and performed several examinations. However, it was confirmed that a small amount of FXI contaminated the purified IgG and impaired the intended examinations. In spite of such difficulties, we obtained

the information concerning characteristics of this antibody by use of synthetic chromogenic substrate assay for factor VIII and factor IX. Combined use of these assays further suggested that this antibody was directed against FXI.

【Conclusion】 In the present study we revealed that a high titer of autoantibodies which bind against FXI existed in the patient's plasma and led to complicated laboratory results.

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Strong TCR stimulation promotes the demethylation of Foxp3 CNS2 in regulatory T cells induced in vitro

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Foxp3 is the master transcriptional regulator of regulatory T cells（Tregs）, and the stabilization of Foxp3 expression is regulated by the demethylation of conserved non-coding sequence 2（CNS2）in the Foxp3 locus. Recent studies have shown that TCR stimulation is required for the demethylation of Foxp3 CNS2 during Treg development. However, the relationship between the strength of TCR stimulation and the demethylation of Foxp3 CNS2 remains unclear. To address this issue, we compared the frequency of demethylation of the Foxp3 CNS2 among in vitro-induced Tregs（iTreg）that had received a range of TCR stimulation during their development. We found that the frequency of demethylation of the Foxp3 CNS2 was increased with increased TCR stimulation strength, whereas CD28 stimulation had only a limited effect. Mechanistically, the binding of Tet2, a member of the TET family of enzymes involved in DNA demethylation, on the Foxp3 CNS2 was increased by strong TCR stimulation. Furthermore, compared with iTreg induced by weak TCR stimulation, iTreg induced by strong TCR stimulation maintained Foxp3 expression both in vitro and in vivo. These data indicate that the strength of TCR stimulation is a key factor for induction of the demethylation of Foxp3 CNS2 and the generation of stable Tregs.

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Calmodulin-like skin protein suppresses hydrogen peroxide- or ultraviolet-induced increase in senescence associated beta-galactosidase in keratinocytes

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Calmodulin-like skin protein（CLSP）is a secreted peptide that is restrictedly produced in skin keratinocytes and some related epithelial cells. It has been previously shown that CLSP is recruited via blood stream into the central nervous system where it likely exerts neuroprotective effect against toxicity related to Alzheimer's disease（AD）by binding to the heterotrimeric humanin receptor and activates intracellular survival signaling. However, it remains to be elucidated whether secreted CLSP has some biological activities outside the central nervous system. In the current study, using hydrogen peroxide（H_2O_2）- and ultraviolet（UV）-induced senescence models of primarily cultured skin keratinocytes, we have addressed to the question as to whether CLSP is involved in senescence of skin keratinocytes. We first found that CLSP expression was potentiated by the treatment with H_2O_2 and the exposure to UV in keratinocytes. Furthermore, the co-incubation with recombinant CLSP reduces Senescence-associated beta-galactosidase-positivity in keratinocytes that is induced by the treatment with H_2O_2 and the exposure to UV. These results suggest that CLSP may function as a senescence-suppressing factor for keratinocytes.