論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	XING YAN　FANG
論文題名 Title	RNA editing of AZIN1 coding sites is catalyzed by ADAR1 p150 after splicing (AZIN1コード領域に生じるRNA編集は、スプライシング後にADAR1 p150によって触媒される)

論文内容の要旨

〔目　的(Purpose)〕

Adenosine-to-inosine (A-to-I) RNA editing is a post-transcriptional modification, which is catalyzed by adenosine deaminase acting on RNA1(ADAR1) and ADAR2. ADAR1 is expressed as two isoforms: a type I interferon (IFN)-inducible ADAR1 p150 and ADAR1 p110. ADAR1 p150 is predominantly expressed in the cytoplasm and enriched in lymphoid organs, whereas nuclear ADAR1 p110 and ADAR2 are abundant in the brain, all of which recognize double-stranded RNAs (dsRNAs) as targets. Although its frequency is quite rare, RNA editing sometimes occurs in coding regions, affecting protein functions by altering amino acid sequences, given that inosine is interpreted as guanosine by the translational machinery. Coding sites are generally edited in the nucleus, given that the corresponding exon forms a dsRNA structure with an adjacent intron. This means that the processing must be executed by nuclear ADAR1 p110 or ADAR2 before splicing. Therefore, certain coding sites, which are edited by cytoplasmic ADAR1 p150, remain unidentified. However, we previously found that RNA editing at two coding sites in antizyme inhibitor 1 (AZIN1) protein is sustained in *Adar1 p110/Aadr2* double knockout mice. Thus, this study was aimed to elucidate the molecular mechanisms underlying RNA editing of AZIN1mRNA.

〔方法ならびに成績(Methods/Results)〕

We first investigated whether two RNA editing sites of the *Azin1* gene were responsible for type I IFN treatment *in vitro*. For this purpose, we stimulated mouse macrophage-like Raw 264.7 cells with IFNβ1 to induced ADAR1 p150 and found that the editing levels of these two sites significantly increase. Second, we transfected a plasmid containing enhanced green fluorescent protein (EGFP)-tagged ADAR1 p110, p150 or ADAR2 into *Adar1/Adar2* double-knockout (A1/A2 dKO) Raw 264.7 and human HEK293T cells and found that AZIN1 coding sites are editable *in vitro* only by ADAR1 p150 at the mature mRNA stage, which is conserved between human and mouse cell lines. Thirdly, to identify the dsRNA structure required for Azin1 RNA editing, we expressed mCherry-fused reporter mRNA in Raw 264.7 cells and found that a dsRNA structure is formed between the corresponding exon and the downstream exon after the splicing of the the intron intervening between these two exons. Fourthly, we expressed various EGFP-tagged ADAR1 mutants in A1/A2 dKO Raw 264.7 cells and found that the intracellular localization of ADAR1, but not binding capacity to Z-RNA, is critical for regulating Azin1 RNA editing. Lastly, to investigate whether ADAR1 150 is responsible for Azin1 RNA editing *in vivo*, we created *Adar1 p150*$^{-/-}$ mice and found that RNA editing at Azin1 coding sites was absent in these mutant mice.

〔総　括(Conclusion)〕

The results obtained in this study collectively indicate that AZIN1 coding sites are edited by ADAR1 p150 after splicing *in vitro* and i*n vivo*. This is the first case in which coding sites are editable by ADARs after splicing, modulating the degree of recoding via regulation of ADAR1 p150 expression. RNA editing of Azin1 coding sites is known to affect intracellular localization of AZIN1, altering the binding partners. In addition, multiple lines of evidence suggest that RNA editing of AZIN1 coding sites is increased in cancers, which is most likely a response to type I IFN production from the chronic inflammatory environment of cancers in addition to a possible increase in *ADAR1* copy numbers.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

RNA中のアデノシンをイノシンへと置換するRNA編集は、稀にタンパク質コード領域に生じることがある。この場合、当該アミノ酸配列を置換し、タンパク質の性質を変容させる可能性があることから、生理学的に重要である。触媒を担当するRNA編集酵素ADAR1とADAR2は2本鎖RNAを標的とし、通常は上下流のイントロンと当該エクソンが2本鎖RNAを形成することが必須のため、スプライシング前に核内でRNA編集が生じる。今回、Xing氏らは、AZIN1と呼ばれるがん関連タンパク質のコード領域に生じるRNA編集が、スプライシング後に生じることを発見した。これは、当該エクソンと下流のエクソン間で2本鎖RNA構造が形成されるためで、間に挟まれたイントロンは核内でのRNA編集を阻害する役割を果たしていることを見いだした。細胞質局在型でインターフェロン誘導型のADAR1 p150が責任酵素であることも突きとめた。スプライシング後のコード領域RNA編集はこれまで報告例がなく、極めて重要な発見と言える。以上から、本研究内容は、学位に値するものと認める。