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RNA editing of *SLC22A3* mRNAs: causative relevance to familial ESCC?

Kazuko Nishikura

The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19104, USA

Abstract

A new study reveals an involvement of *SLC22A3* in the development of familial oesophageal squamous cell carcinoma (ESCC). Reduced expression of *SLC22A3* is detected not only in ESCC tumours but also in non-tumour tissues of patients with familial ESCC. Interestingly, adenosine-to-inosine editing of *SLC22A3* mRNA is proposed to drive early tumour invasion and metastasis, by inhibiting *SLC22A3* expression.

Oesophageal squamous cell carcinoma (ESCC) is one of the leading causes of death from cancer worldwide¹. The geographically concentrated distribution and familial aggregation of this cancer among high-risk Northern Chinese people has indicated a possible genetic susceptibility. Familial oesophageal ESCC often shows earlier onset and worse prognosis compared with non-familial ESCC. However, the genetic basis underlying the molecular pathogenesis has yet to be established.

RNA editing refers to post-transcriptional modification of RNA nucleotides. One type of RNA editing converts adenosine to inosine specifically in double-stranded RNAs (dsRNAs). This A-to-I RNA editing is catalysed by ADARs (adenosine deaminase acting on RNA, also known as double-stranded RNA-specific adenosine deaminase), of which ADAR1 and ADAR2 are enzymatically active². Association of elevated A-to-I RNA editing with almost all types of cancer has been revealed through analysis of The Cancer Genome Atlas data³. However, how A-to-I editing contributes to oncogenesis remains largely unknown.

SLC22A3 (solute carrier family 22 member 3, also known as *OCT3*) encodes a cation transporter that has a critical role in the transport of drugs and cellular detoxification⁴. An increased risk of prostate and colon cancer associated with the locus harbouring *SLC22A3* has been previously reported^{5,6}. A new study by Fu and colleagues⁷ identifies suppression of *SLC22A3* as a major cause of early onset and metastatic progression of familial ESCC. Most interestingly, they report that A-to-I editing of *SLC22A3* mRNA by ADAR2, which results in recoding of asparagine 72 to aspartate (*SLC22A3*-Asn72Asp), also contributes to genesis of familial ESCC.

“...it seems that suppression of the *SLC22A3* gene is the primary mechanism of familial ESCC pathogenesis”

Competing interests statement

The author declares no competing interests.

Gene expression profiling studies of five patients with familial ESCC from an ESCC high-risk area of China identified 113 upregulated and 67 downregulated genes. Gene ontology analysis of these genes indicated enrichment of biological processes for response to organic substances and response to extracellular stimuli, leading to further focused studies on *SLC22A3*. *SLC22A3* mRNA levels were reduced not only in tumours but also in normal tissues, indicating an altered genetic background and also one possible reason for early development of cancer in these patients. Pyrosequencing of cDNA from 76 familial and 145 sporadic ESCCs detected higher levels of A-to-I editing of *SLC22A3* mRNAs (A-to-G in the cDNA sequence) in non-tumour tissues of familial (~40%) than sporadic ESCCs (~2%). The inverse relation between this A-to-I editing frequency and *SLC22A3* mRNA levels was confirmed only for non-tumour tissues of familial ESCCs. Moreover, *SLC22A3* mRNA editing correlated with lymph node metastasis.

The A-to-I editing of *SLC22A3*, mediated by ADAR2, occurred at A261 of exon 1 and resulted in an Asn72Asp substitution in the protein. A previous genome-wide association study identified *ADAR2* (also known as *ADARBI*) located at 21q22.3 as an ESCC susceptibility locus⁸; reanalysis of this data revealed one single-nucleotide polymorphism within intron 1 of *ADAR2* that was associated with the familial ESCC cases, pointing to *ADAR2* as a susceptibility gene for familial ESCC. The expression of *ADAR2* was indeed increased in non-tumour tissues from patients with familial ESCC compared with patients with sporadic ESCC. Ectopic expression of edited *SLC22A3* in normal oesophageal cell lines decreased cell adhesion, whereas the unedited isoform promoted adhesion. Furthermore, the edited *SLC22A3* increased cell migration and invasion, whereas the unedited isoform inhibited these properties. When a mixture of edited and unedited isoforms was tested, metastatic phenotypes appeared progressively, dependent on the ratio of edited versus unedited *SLC22A3* isoforms. The tumorigenic properties seem to correlate clearly with the level of *SLC22A3* mRNA editing, which leads to a loss of SLC22A3 function. The unedited SLC22A3 isoform suppressed the epithelial-to-mesenchymal transition *in vitro* in transfected cells and metastasis *in vivo* in a lymph node metastasis mouse model. Finally, stable knockdown of *SLC22A3* promoted motility of normal oesophageal cells, further supporting the hypothesis that loss of SLC22A3 function contributes to the early development and progression of familial ESCC.

Co-immunoprecipitation and mass spectrometry analysis identified 27 proteins that interact with the unedited *SLC22A3* isoform but not with the edited version. α -Actinin 4 (ACTN4), which is known to be involved in cell motility, was the highest scoring SLC22A3-interacting protein. The interaction of the unedited — but not the edited — SLC22A3 isoform with ACTN4 was further confirmed by a pull-down assay. ACTN4, an actin-binding protein, promotes formation of filopodia by crosslinking actin filaments into bundles. ACTN4-mediated actin crosslinking is inhibited by the unedited SLC22A3 isoform (FIG. 1a), whereas suppression of SLC22A3 (FIG. 1b) and expression of the edited isoform (FIG. 1c) seems to increase the interaction between F-actin and ACTN4, thereby promoting their cross linking. Computer modelling predicted that the aspartate residue introduced by RNA editing resides in the third α -helical extracellular region of SLC22A3-Asn72Asp (FIG. 1a). Accordingly, the authors suggested this extracellular region of the unedited SLC22A3 isoform as the ACTN4 binding interface. However, modelling also revealed marked

conformational changes in the cytoplasmic *N*-terminus region of the SLC22A3-Asn72Asp isoform. In view of ACTN4 interaction with actin filaments, it is also possible that ACTN4 binds to the *N*-terminus region of the unedited SLC22A3 isoform at the cytoplasmic side (FIG. 1a). In any case, the ACTN4 binding interface of the unedited SLC22A3 isoform is probably disrupted in the edited isoform (FIG. 1c). Knockdown of ACTN4 reduced invasiveness of cells overexpressing the edited SLC22A3 isoform. Cell motility induced by the ACTN4-mediated mechanism is regulated by the Rho-family GTPase CDC42 at the cell periphery. Knockdown of ACTN4 reduced filopodia formation and CDC42 expression in cells overexpressing SLC22A3-Asn72Asp.

The findings of Fu *et al.*⁷ suggest that the misregulation of *SLC22A3* predisposes individuals of familial ESCCs to a high risk of developing ESCC early in life. Repression of SLC22A3 also accelerates the cell motility of oesophageal epithelial cells by enhancing ACTN4-mediated filopodia formation, leading to promotion of the early malignant progression of ESCC. As proposed by Fu *et al.*⁷, SLC22A3 seems to act as a metastasis suppressor by directly inhibiting ACTN4. A-to-I editing of *SLC22A3* mRNAs by ADAR2, and resultant synthesis of SLC22A3-Asn72Asp, seems to be capable of suppressing the metastasis-inhibiting function of the unedited SLC22A3 isoform, accelerating the progression of familial ESCC.

These findings certainly establish the molecular pathogenesis of familial ESCC mediated by repression of *SLC22A3* function and its unique characteristics, such as early onset and a progressive disease course. However, several issues remain unresolved. First, the molecular mechanism for reduced *SLC22A3* mRNA levels in familial ESCC is not known. The possibility of epigenetic regulation was indicated, but post-transcriptional regulation such as mRNA decay and translational control are also possible. Second, the reason for increased expression of ADAR2 specifically in familial ESCCs is not presented. Perhaps, there is some genetic alteration such as amplification of the 21q22.3 loci harbouring *ADAR2*? Finally, and most importantly, what is the relative importance of *SLC22A3* mRNA editing versus the reduction of *SLC22A3* expression in familial ESCC pathogenesis? The A-to-I editing frequency is relatively low, in the range of ~40% at most, and in some cases no editing was detected at all. Thus, it seems that suppression of the *SLC22A3* gene is the primary mechanism of familial ESCC pathogenesis. Perhaps *SLC22A3* mRNA editing contributes by decreasing the function of the remaining low level unedited *SLC22A3* isoform.

In the past few years, a positive contribution of A-to-I RNA editing to many cancers has been reported³. However, a negative contribution via suppression of metastasis has also been reported⁹. Certainly more studies are required to further define the roles of RNA editing mediated by ADARs in cancer and metastasis.

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Figure 1. Suppression of SLC22A3 and A-to-I editing of *SLC22A3* mRNA by ADAR2 promotes early onset and progression of familial ESCC

a | Unedited solute carrier family 22 member 3 (SLC22A3) inhibits the metastasis-promoting function of α -actin 4 (ACTN4). **b** | In patients with familial oesophageal squamous cell carcinoma (ESCC) with absence or reduced expression of unedited SLC22A3, ACTN4 crosslinks actin filaments and converts them into actin bundles, promoting filopodia formation and metastasis. **c** | The edited SLC22A3 isoform is unable to interact with ACTN4 and also promotes filopodia formation and metastasis. ADAR2, adenosine deaminase acting on RNA 2 (also known as ADARB1); D, aspartate; N, asparagine.