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General Summary

Our research have focused on biological significance of regulating cellular polyamines, in particular through a polyamine-regulating protein "antizyme". Polyamines are ubiquitous biogenic amines that are essential for cell proliferation and related to various phenomena such as differentiation, development, cancer, and autophagy. The latest works revealed that polyamines have significant effects on longevity, memory and arteriosclerosis. Three major polyamines, putrescine, spermidine and spermine, are present in mammalian cells. When cellular polyamines increase, AZ is induced through translational frameshift. AZ binds to ornithine decarboxylase (ODC), a key enzyme for polyamine biosynthesis, and inhibits the enzymatic activity and accelerates degradation of the enzyme protein. Thus AZ provides the feedback regulation for the cellular polyamine levels. Mammalian cells express three members of the AZ family (AZ1-3) and each AZ is likely to have specific function. In addition, AZ is regulated by antizyme inhibitors (AZINs) which are homologous to ODC but lack the enzymatic activity.

Research Activities

Functional Significance of Interaction between MYCN and AZ2 in neuroblastoma cells
We have previously found that AZ2 interacts with c-Myc and accelerates its degradation in ubiquitin-independent manner. We further found that AZ2 also interacts Myc protein family MYCN which are highly expressed in neuroblastoma cells, and accelerates its degradation like c-Myc. To clarify the involvement of AZ2 in neuroblastoma cell growth, We performed soft-agar colony formation assay using AZ2 knocking down neuroblastoma cell line, BE(2)-C cells with siRNA. Knocking down of AZ2 stabilized the level of MYCN and increased the colony formation more than two-fold, and the size of colonies are larger than that of control cells. These results suggest that AZ2 regulates neuroblastoma cell growth through the degradation of MYCN. We are planning to confirm the tumor formation at the whole body level by xenograft mouse model analysis using AZ2 knocked down BE(2)-C cells.

Analysis of interaction between AZ and ATP citrate lyase
We identified ATP citrate lyase (ACLY), a cytosolic enzyme which catalyzes the production of acetyl-CoA that is used for lipid anabolism or acetylation of cellular components by the screening for AZ-binding proteins. We recently reported that AZ1 and AZ2 bind to and activate ACLY in cancer cells. This function of AZ was confirmed not only in cancer cells but also in adipose like 3T3-L1 cells. It is known that polyamines and ACLY are indispensable for differentiation of adipocytes. We are continuing the study for the cross-

talk between polyamines and ACLY through the function of AZ. Aside from this, considering the possibility that acetyl-CoA which is produced by ACLY facilitates acetylation of polyamines and resulting increase of excretion of acetyl-polyamines, we are studying to confirm the hypothesis.

Analyses of physiological roles of Azin1

Antizyme inhibitor 1 (AZIN1) binds to antizyme (AZ) with higher affinity than ODC does, and consequently leads to release of ODC from the AZ-ODC complex. Thus AZIN1 functions as a positive regulator for cellular polyamines. To clarify the biological functions of AZIN1, we established spontaneous immortalized mouse embryonic fibroblasts (MEFs) from wild-type mice (W-MEFs) and mutant mice (M-MEFs), in which expression of AZIN1 is greatly decreased. We performed cell growth, cell division and metabolome analysis using these MEFs. In M-MEFs, polyamine concentration was decreased due to stabilization of AZ which accelerates ODC degradation, and cell growth retardation was observed. In addition, DNA damage was predicted from the observation of binuclear cells with micronuclei. Considering that addition of putrescine could not reduce the number of binuclear cells, AZIN1 may be involved in those phenomenon directly. In metabolome analysis, the metabolites related to DNA synthesis and methylation such as 5-methyl tetrahydrofolate and S-adenosylmethionine were decreased in M-MEFs. We now continue to examine the detail of the role of AZIN1.

Analysis of AZ1 +1 ribosomal frameshift mechanism in a human cell-free translation system

AZ1 mRNA consists of two open reading frames, ORF1 and ORF2. Normally, ORF1 coding product is translated, but when the cellular polyamine level increased, antizyme (ORF2 coding product fused with that of ORF1) is produced by +1 translational frameshift mechanism. It is known that three cis elements, upstream stimulator (50 nucleotide sequence just 5′ of frameshift site), frameshift site (stop codon of ORF1) and pseudoknot structure which consists of about 60 nucleotide downstream to frameshift site on AZ1 mRNA are required for an efficient +1 ribosomal frameshift. We found that AZ1 mRNA lacked all the cis elements still allowed for spermidine induced +1 frameshifting in a HeLa cell extract translation system. This result suggests that the spermidine-induced +1 frameshift occurred in ORF2 mRNA except for pseudoknot structure. To determine the extra frameshifting position in ORF2 mRNA, termination codons were introduced in ORF2 mRNA. Results showed that the efficiency of the +1 frameshift increased in proportion to the length of the sequences in ORF2. To confirm whether this result occurs in mRNA other than AZ1, we designed reporter system to assay frameshifts efficiency of any sequence of interest. The +1 frameshift was observed to occur in many sequences other than AZ1 mRNA in a spermidine-dependent manner. These findings may suggest that polyamine has a potential to shift the reading frame to the +1 direction at any sequence in a HeLa cell-free translation system.

Publications

Murai N, Murakami Y, Tajima A, Matsufuji S. Novel ubiquitin-independent uncleolar c-Myc degradation pathway mediated by antizyme 2. *Sci Rep.* 2018; **8:** 3005.

Yamamoto Y[1]***, Makino T***[2]***, Kudo H***[2]***, Ihn H***[2]***, Murakami Y, Matsufuji S, Fujiwara K***[1]***, Shin M***[1] ***(***[1]***Sojo Univ,*** [2]***Kumamoto Univ).*** Expression and distribution patterns of spermine, spermidine, and putrescinein rat hair follicle. *Histochem Cell Biol* 2018; **149:** 161-7.