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General Summary

Our research has focused on the biological significance of regulating cellular polyamines, in particular through the polyamine-regulating protein antizyme (AZ). Polyamines are ubiquitous biogenic amines that are essential for cell proliferation and are related to various phenomena, such as differentiation, development, cancer, and autophagy. The latest works have shown 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 a translational frameshift. After being induced, AZ binds to ornithine decarboxylase, 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 cellular polyamine levels. Mammalian cells express 3 members of the AZ family (AZ1-3), and each AZ is likely to have a specific function.

Functional significance of interaction between MYCN and AZ2 in neuroblastoma cells
We have previously found that AZ2 interacts with MYCN, which is highly expressed in neuroblastoma and is a poor prognosis factor for patients. AZ2 accelerates MYCN degradation in ubiquitin-independent manner. So far, knocking down AZ2 in neuroblastoma cells with small interfering RNA has increased colony formation in soft agar more than two-fold compared with that in control cells. This year we performed xenograft mouse model analysis with AZ2 knocked down BE (2)-C cells to confirm the tumor formation at the whole-body level. As expected from colony formation assay, 24 days after nude mice had been implanted with AZ2 knocking down cells, tumor volumes had increased 7-fold and weight had increased 4-fold compared with those in control cells. These results strongly indicate that AZ2 is involved in tumor growth in neuroblastoma. We are studying the detailed mechanism of AZ2-mediated suppression of tumor growth and crystal structure analysis of the AZ2-MYCN complex for drug development.

Analysis of interaction between AZ and ATP citrate lyase
We identified ATP citrate lyase (ACLY), a cytosolic enzyme that catalyzes the production of acetyl-CoA and is used for lipid anabolism and the acetylation of cellular components, by screening for AZ-binding proteins. We have recently reported that AZ1 and AZ2 bind to and activate ACLY in cancer cells. Although AZ is a negative regulator of cellular polyamines, how the activation of ACLY by AZ is related to polyamine metabolism is unclear. A likely hypothesis is that acetyl-CoA produced by ACLY from citrate in the cytoplasm facilitates the acetylation of polyamines, and, as a result, the export of intracellular acetyl-polyamines is increased. To confirm this hypothesis, intracellular and extra-

cellular acetyl-polyamines under the culture of ACLY overexpressed EXOD-1 cells (a variant cell line isolated from murine breast cancer cells) were measured and compared with control cells. In ACLY overexpressed EXOD-1 cells, both intracellular and extracellular acetyl-polyamines were significantly increased. This result suggests a relationship of ACLY activity to intracellular polyamine acetylation. We are studying the effects of ACLY or AZ knockdown on the levels of intracellular and extracellular acetyl-polyamine in EXOD-1 cells to clarify the mechanism in detail.

Analyses of physiological roles of AZIN1
Antizyme inhibitor (AZIN) 1 is a catalytically inactive homolog of ornithine decarboxylase which positively regulates cellular polyamines by inhibiting AZ. We have previously shown that AZIN1-deficient mouse embryonic fibroblasts (M-MEFs) have the features of growth inhibition and morphological changes, such as the appearance of cells having multinuclei, a macronucleus, or a micronucleus. To address the cause of these phenomena, we focused on intact M-MEFs (iM-MEFs) and intact W-MEFs (iW-MEFs) having no nucleus morphological changes. We measured the centrosome number in iM-MEFs and iW-MEFs by means of antibodies for gamma-tubulin as the centrosome marker. Centrosome numbers were lower in iM-MEFs than in iW-MEFs. Considering our previous report that AZIN1 colocalizes with Az1 mainly at centrosomes during the period from prophase through late anaphase, these results suggest that the defects of AZIN1 lead to a fault of chromosomal segregation mediated by gamma-tubulin and to morphological changes, such as multinuclei or a macronucleus. Our findings suggest that AZIN1 is an important factor for proper cell-cycle progression.

Translation efficiency affects the sequence-independent +1 frameshifting by polyamine
Using a human cell-free translation system, we have shown that ribosomal +1 frameshifting occurred in both a sequence-independent and a polyamine-dependent manner. To gain insight into the mechanism of this +1 frameshifting, we investigated the relationship of efficiencies of +1 frameshifting to translation. To evaluate the translation efficiency of messenger RNA sequences, we used a codon adaptation index (CAI) for human genes. The CAI is an effective measure of synonymous codon usage bias. The index is evaluated within the range of 0 to 1. A CAI range of 0.8 to 1 represents high translation efficiency. The CAI range of transcription factor + 36 nucleotides (TF+36nt) (TCCTTCTGCTCTTTCAGCCAACTTATTCTACTCCGACGATCGGCT, from the readthrough region of the human AZ1 gene) is 0.50, and this value represents a low translation efficiency. Substitution of 7 codons in the TF+36nt sequence with synonymous codons of higher CAI increases the CAI value to 0.86 (TCCTTCTGCTCTTTCAGCCAgCTcATTCTgCTgCGgCGgTCcGCT, substituted nucleotides were indicated by lowercase letters). These substitutions reduced the efficiency of +1 frameshifting compared with the original TF+36nt sequences. Conversely, substitution of 6 codons in CAT45 (AACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTCACGATG, from a bacterial chloramphenicol acetyltransferase gene), which has a high CAI (0.86) into synonymous codons of a lower CAI (0.62) (AAtGTtGCgAATATGGACAAtTTCTTCGCgCCgGTTTTCACGATG, substituted nucleotides were indicated by lowercase letters),

increased the probability of +1 frameshifting as compared with the original CAT45 sequences. These results suggest an inversed correlation between the efficiencies of sequence-independent polyamine-induced +1 frameshifting and translation.

Publications

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