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General Summary

The aims of our research are to clarify the mechanism and biological significance of polyamines and their elaborate regulatory system and to develop polyamine-related medical applications. Polyamines are ubiquitous biogenic amines and are essential for cell proliferation. They are related to various phenomena, such as differentiation, development, cancer, and autophagy. The latest studies have shown that polyamines have effects on longevity, memory, and arteriosclerosis. Three major polyamines—putrescine, spermidine, and spermine—are present in mammalian cells. Ornithine decarboxylase (ODC) is a key enzyme of polyamine biosynthesis in mammalian cells. The role of ODC is to convert ornithine to putrescine, which in turn leads to spermidine and spermine. To be degraded, ODC interacts with antizyme (AZ). Three AZ isoforms (AZ1-3) are present in mammals. The AZs are expressed by translational frameshifting that is induced by polyamines and negatively regulate cellular polyamines. Cellular polyamine contents are maintained by the feedback mechanism involving AZ. The AZs are further regulated by proteins termed antizyme inhibitors (AZINs).

Research Activities

The role of antizyme 2 in neuroblastoma tumor growth

We have previously found that polyamine regulating protein AZ2 accelerates the ubiquitin-independent degradation of protooncogene MYCN which is a factor for the poor prognosis of patients with neuroblastoma. Last year, we revealed, with a colony formation assay in soft agar and xenograft mouse model experiment, that knockdown of AZ2 facilitates neuroblastoma tumor growth. This year, comprehensive analysis was performed with RNA sequencing of gene expression in an AZ2 knockdown neuroblastoma cell line. In AZ2 knockdown cells, mRNA expression of the Fos and Jun families, which are strongly related to cell growth, differentiation, and survival, were increased, whereas expression of effector and initiator caspases, which are deeply involved in apoptosis, were significantly decreased. These results suggest that AZ2 knockdown enhances cell growth in a neuroblastoma cell line by upregulating cell growth-related genes and downregulating apoptosis-related genes.

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, which is used for lipid anabolism or acetylation of cellular components by the screening for AZ-binding proteins. We have recently reported that AZ1 and AZ2 bind to and activate ACLY in cancer cells. However, the significance of ACLY activity on

polyamine metabolism was unclear, despite AZ being a negative regulator of cellular polyamines. We are continuing the study for the crosstalk between polyamines metabolism and ACLY through the function of AZ. The 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 extracellular acetyl-polyamines of ACLY-overexpressed cells were measured. Contrary to expectations, both intracellular and extracellular acetyl-polyamines were significantly increased. These results indicate that a factor other than ACLY is needed for the export of acetylated polyamines.

Translation efficiency affects the sequence-independent +1 ribosomal frameshifting by polyamines

Synthesizing the functional AZ protein requires transition of the reading frame at the termination codon. We have reported that spermidine has the potential to shift the reading frame in the +1 direction in any sequence using a human cell-free translation system. The probability of this promiscuous +1 frameshifting by spermidine has an inverse correlation with the efficiency of translation. This sequence-independent +1 frameshifting can also be induced by putrescine and spermine, although the dose required for +1 frameshifting was quite different from that required by spermidine. These results suggest that polyamines potentially induce the sequence-independent +1 frameshifting. The polyamine-dependent +1 frameshifting was also detected in translation with *in vitro* transcribed RNA templates in place of DNA templates, supporting that +1 frameshifting in this *in vitro* protein expression system occurred during translation.

Polyamines involved in respiratory function

The extracellular polyamine concentration is about 0.1% to 1% of the intracellular polyamine concentration. Because polyamines are present in all organisms and in all cells, most polyamine research has focused on the intracellular function of polyamines. We found that polyamines are present in alveoli. To investigate the effects of polyamines on respiratory function, the effects of polyamines were examined with a lavage model established as a rat model of acute respiratory distress syndrome. We found that polyamines administered to the alveoli improve lung compliance, arterial blood oxygenation, and lung aeration. Many patients with acute respiratory distress syndrome die in the absence of effective treatment. Now we are continuing the verification toward the practical application to patients.

Publications

Katagiri S, Hosono K, Hayashi T, Murai N, Wake E, Miyata I, Mizobuchi K, Kurata K, Matsuura T, Nakano T, Hotta Y. Novel biallelic splice-site BBS1 variants in Bardet-Biedle syndrome: a case report of the first Japanese patient. *Doc Ophthalmol.* 2020 Aug; **141**(1): 77-88. doi: 10.1007/s10633-020-09752-5. Epub 2020 Jan 29. PubMed PMID: 31997113.

Oguro A, Shigeta T, Machida K, Suzuki T, Iwamoto T, Matsufuji S, Imataka H. Translation efficiency affects the sequence-independent +1 ribosomal frameshifting by polyamines. *J Biochem*. 2020 Mar 17. pii: mvaa032. doi: 10.1093/jb/mvaa032. [Epub ahead of print] PubMed PMID: 32181810.