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General Summary

Polyamines (putrescine, spermidine, and spermine) are ubiquitous biogenic amines present in all cells. The cellular polyamines mainly bind to RNA and DNA to stabilize them and to control their function and, therefore, are indispensable for cell proliferation. Polyamines also regulate various cellular processes, such as apoptosis and signaling through the *N*-methyl-d-aspartate receptors. The concentration of cellular polyamines is regulated by a family of proteins, termed antizymes (AZs). These AZs are expressed and induced through a unique mechanism, translational frameshifting, and negatively regulate cellular polyamine levels by accelerating the degradation of ornithine decarboxylase, a key enzyme for polyamine biosynthesis, and by inhibiting cellular polyamine uptake. Three AZ isoforms (AZ1–3) are conserved among mammals. Our goal is to clarify the mechanism and biological significance of the polyamine regulatory system, particularly the roles of the proteins involved.

Research Activities

Hematopoietic disturbance of AZ1-knockout mice

AZ1-deficient mouse embryos show hematopoietic disturbances and consequent severe anemia resulting in partial embryonic death. Our previous results suggested that the early erythroid progenitors are reduced in the livers of AZ1-deficient embryos. To further determine the hematopoietic steps affected, we analyzed embryonic liver cells with fluorescence-activated cell sorting (FACS) and colony formation assay. The FACS analysis revealed increases in hematopoietic stem cells and the early erythroid progenitor cells in the livers of AZ1-knockout embryos, indicating that the migration of early hematopoietic cells into the fetal liver is not impaired. On the colony formation assay, the mixed colonies decreased in the livers of AZ1-knockout embryos. The mixed colony corresponds to the common myeloid progenitor (CMP), a precursor of erythroid and myeloid lineages, and to the multipotent progenitor (MPP), a further precursor of CMP. Thus, the results suggest that the differentiation either from hematopoietic stem cells to MPP or from MPP to CMP is impaired in AZ1-deficient mice.

Defensive role of AZ1 against over-intake of polyamines

To assess the defensive role of AZ1 against over-intake of polyamines, adult AZ1-deficient knockout mice and control mice were fed composite meals containing a high dose of polyamines (25 times that of normal meals) for 2 weeks. As previously observed, AZ1-deficient mice tended to be leaner than the control mice at the start of the feeding experiment, but the changes in body weight did not differ between the groups

during the experiment. Blood levels of putrescine, spermidine, and spermine were higher in the AZ1-deficient mice than in the control mice before feeding, but spermine levels decreased markedly in the AZ1-deficient mice after day 7 of the high-polyamine feeding. Urine excretion of polyamines increased in both AZ1-deficient and the control mice fed high-polyamine meals until day 7. On day 14, polyamine excretion was still increasing in the AZ1-deficient mice but had decreased in control mice. Urinary levels of acetylated polyamines kept increasing with the high-polyamine diet, particularly in AZ1-deficient mice. These results suggest that, in wild-type mice absorption of polyamines is repressed by AZ1 when high doses of polyamines are ingested, whereas in AZ1-deficient mice, the lack of repression is compensated for by the acetylation of polyamines and the suppression of spermine synthesis.

AZ2 accelerates c-Myc degradation

We have found with yeast two-hybrid analysis that AZ2, but not AZ1, binds to cerebellar degeneration-related protein 2, which is a c-Myc-interacting protein implicated in paraneoplastic cerebellar degeneration. During the experiments, we found through pull-down assays that AZ2 directly interacts with c-Myc in cultured mammalian cells. Both AZ2 and c-Myc, tagged with fluorescent proteins, co-localized in the nucleus. AZ1 weakly interacted and partially co-localized with c-Myc. We then investigated the effect of AZ2 on the degradation of c-Myc. Overexpressed, tagged c-Myc, as well as the endogenous c-Myc, was degraded more rapidly in the cells transfected with AZ2 than in control cells. The acceleration of c-Myc degradation was inhibited by a proteasome inhibitor, MG132. The effect of AZ2 was not observed in a cell-free system. Finally we found that the addition of putrescine to cultured mammalian cells accelerated the degradation of endogenous c-Myc. These results suggest that an increase in cellular polyamines induces AZ2 and negatively regulates c-Myc through the acceleration of degradation by cellular 26S proteasomes.

Analyses of RNA aptamers against spermine

RNA aptamers are functional RNAs that have a high affinity for their biomolecular targets and can distinguish similar chemical structures. Last year we isolated RNA aptamers with a high affinity against spermine (spermine aptamers). To determine the spermine-binding regions of the aptamers, we performed mutational and domain exchange analysis between a spermine aptamer and another RNA aptamer that has a similar conformation but does not bind spermine. Multiple binding regions for spermine were determined on the spermine aptamer. Kinetic analysis between the spermine aptamer and spermine were also performed with the surface plasmon resonance technique. However, we could not determine the binding kinetics because of nonspecific interaction with each electrostatic region. Other methods are needed for analyzing the binding between the spermine aptamers and spermine.

A protein enhancing AZ frameshifting

We have been seeking cellular proteins that modulate translational frameshifting and found that transient expression of heterogeneous nuclear ribonucleoprotein A1-like

(hnRNP A1L) enhances the efficiency of frameshifting by 2 times over that of a co-expressed dual-luciferase reporter construct encoding the AZ1 frameshifting signal. A close homologue of hnRNP A1L, hnRNP A1, which is known to interact with various nucleic acids both in the cytosol and the nucleus, had no effect on the frameshift efficiency. The proteins hnRNP A1 and hnRNP A1L comprise 320 amino acid residues and have 11 amino acid differences. The different amino acids are spread over the molecules and on some domains, such as 2 RNA-recognition motifs, the RGG box and the M9 signal sequence. Domain substitution analysis with a series of chimeric constructs between hnRNP A1 and A1L showed that the 2 RNA recognition motifs of hnRNP A1L are important for the frameshifting-enhancing effect.

Publications

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