Department of Molecular Biology

Senya Matsufuji, *Professor*
Noriyuki Murai, *Assistant Professor*
Akihiro Oguro, *Assistant Professor*

General Summary

Polyamines (putrescine, spermidine, and spermine) are ubiquitous biogenic amines that bind mainly to nucleic acids and are essential for cell proliferation. Ornithine decarboxylase (ODC) is a key enzyme of polyamine biosynthesis in mammalian cells. ODC converts ornithine to putrescine, which in turn leads to spermidine and spermine. ODC is degraded by interaction 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 a feedback mechanism involving AZ. The AZs are further regulated by proteins termed antizyme inhibitors (Azins). Cancer cells generally contain elevated levels of polyamines. Our goal is to clarify the mechanism and biological significance of the elaborate regulatory system and to develop polyamine-related research or diagnostic tools.

Research Activities

Role of AZ2 in c-MYC degradation
We have previously identified AZ2 as a c-MYC-associating protein that colocalizes with c-MYC in the nucleus and nucleolus and accelerates c-MYC degradation by the proteasome in an antizyme-dependent and ubiquitin-independent manner. We successively elucidated the effects of AZ2 on c-MYC degradation in the nucleolus. The overexpression of the nucleolar protein nucleophosmin 1 (NPM1) has been reported to increase the nucleolar localization of c-MYC and the rate of its degradation. Under this condition, we investigated the effects of knockdown and overexpression of AZ2 on c-MYC degradation in the cells. The knockdown of AZ2 suppressed degradation of endogenous c-MYC. In contrast, overexpression of AZ2 increased the rate of degradation of both wild-type c-MYC and a nonubiqutinated mutant of c-MYC (T58A), which can be degraded in the presence of NPM1. These results suggest that AZ2 accelerates the degradation of c-MYC in the nucleolus in an ubiquitin-independent manner.

Analysis of interaction between AZ2 and ATP citrate lyase
A cytoplasmic enzyme, ATP citrate lyase (ACLY), generates acetyl-coenzyme A from mitochondria-derived citrate and is important for fatty acid synthesis and histone acylation. We have previously identified ACLY as an AZ2-interacting protein and showed that ACLY can interact with both AZ1 and AZ2 and that AZs accelerate ACLY activity *in vitro*. We further investigated mechanism for activation of ACLY by AZs. In cells, induction of AZs by polyamines increased ACLY activity, whereas knockdown of AZ suppressed ACLY activity. The enzyme ACLY is known to be activated by Ser455 phosphor-

ylation, but AZs did not change the phosphorylation at this residue. These results indicate that AZs activate ACLY through novel mechanisms. We are also developing a method for measuring ACLY activity with mass spectrometry and stable isotope-labeled citrate.

Characterization of hematopoietic stem cells in AZ1 knockout mice

We previously showed that the knockout of the gene *AZ1* in mouse increases tissue polyamine levels, reduces the numbers of hematopoietic progenitor cells of multiple lineages (erythroid, granulocyte-monocyte, and common myeloid), and possibly increases the number of hematopoietic stem cells (HSCs) with lowered activity. To discriminate whether the activity of HSCs are generally decreased or only a subpopulation of HSCs are affected, we measured the activity of individual HSCs with limited dilution in transplantations in which the number of donor fetal liver cells per recipient animal was reduced as much as possible. Four months after the transplantation, the reconstituted peripheral blood cells were analyzed in mice engrafted with $AZ1^{+/+}$ HSCs or $AZ1^{-/-}$ HSCs. Some $AZ1^{-/-}$ HSCs-engrafted recipients exhibited a HSC multipotency comparable to that in $AZ1^{+/+}$ HSCs-engrafted recipients, while the other recipients barely had the myeloid progeny from $AZ1^{-/-}$ HSCs. These results indicate heterogeneity in the multipotency of the $AZ1^{-/-}$ HSC pool.

Analyses of physiological roles of Azin1

To address the regulatory roles of Azin1, we analyzed mouse embryonic fibroblasts (MEFs) from a mutant mouse in which expression of Azin1 is greatly diminished. It was noted that the mutant MEF contained many small-shaped cells (～30% of total cells). The activity of ODC in G2/M-synchronized mutant MEFs was only 3.3% of that in the wild-type MEFs, and that induced by cell dilution in the mutant MEFs was 23% of that in the wild-type MEFs. These results suggest the physiological importance of Azin1 for normal cell growth. Next, we compared the expression pattern of proteins in the 2 MEFs. We found some interesting proteins, including ACLY, being differentially expressed. We also analyzed metabolite profiling of the 2 MEFs by capillary electrophoresis-mass spectrometry. Strong metabolic abnormality in polyamine and methionine metabolism was noted in the mutant MEFs.

Analysis of AZ frameshifting mechanism with human PURE system

The human PURE system is an *in vitro* translation system reconstituted with purified human translational factors. A merit of this system is the ease with which specific factors, such as polyamines, can be added or removed or both. Using this system, we are analyzing the molecular mechanism of ribosomal frameshifting of AZ expression. A frameshift product of human AZ1 was synthesized in the system in a polyamine-dependent manner, although the efficiency was still low. We started several experiments in the system to reveal the cis-acting elements or trans-acting factors or both participating in AZ frameshifting.

Interaction analysis of spermine-binding RNA aptamers with spermine

Aptamers of RNA have the potential for both clinical and research applications. In partic-

ular, aptamers are useful for exploring RNA-binding sequences and structures for target molecules. We are revealing general polyamine-binding RNA sequences and structures by analyzing polyamine-binding sites on isolated RNA aptamers. We have isolated a spermine-bindng aptamer. To improve the data of binding affinity last year, we continued interaction analyses using isothermal titration calorimetry and detected the specific 1:0.85 interaction between spermine and this aptamer. With this result, the dissociation constant was estimated to be 27.2 μM. Furthermore, resonances of imino protons in basepairs and H5-H6 cross-peaks of pyrimidine were monitored with nuclear magnetic resonance spectroscopy to investigate the effect of spermine on the tertiary structure of the aptamer. In the presence of spermine, several resonances in these spectra had disappeared or shifted. Interestingly, the affected bases by spermine were positioned on a wide range in this aptamer. We further investigated a thermal stability of the aptamer with or without spermine by UV melting. The melting temperature was shifted to a higher degree in the presence of spermine. These results suggest that spermine induces a dynamic conformational change of this aptamer and stabilizes the aptamer-spermine complex.

Publications

Matoba K, Kawanami D, Tsukamoto M, Kinoshita J, Ito T, Ishizawa S, Kanazawa Y, Yokota T, Murai N, Matsufuji S, Utsunomiya K. Rho-kinase regulation of TNF-α-induced nuclear translacation of NF-κB Rel/p65 and M-CSF expression via p38 MAPK in mesangial cells. *Am J Physiol Renal Physiol*. 2014; **307:** F571-80.

Hirata Y, Murai N, Yanaihara N, Saito M, Saito M, Urashima M, Murakami Y, Matsufuji S, Okamoto A. MicroRNA-21 is a candidate driver gene for 17q23-25 amplification in ovarian clear cell carcinoma. *BMC Cancer*. 2014; **14:** 799.

Reviews and Books

Murai N. Antizyme. In: Kusano T, Suzuki H, editros. Polyamines. Tokyo: Springer Japan; 2015. p. 91-9.