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. Cellular polyamine contents are maintained by a feedback mechanism involving the key regulatory proteins antizymes (AZs). The AZs are expressed by translational frameshifting that is induced by polyamines and negatively regulate cellular polyamines. Three AZ isoforms (AZ1-3) are present in mammals. 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 ubiquitin-independent manner. To clarify the significance of this interaction in the nucleus and nucleolus, we investigated the effects of knockdown of AZ2 or c-MYC on the subcellular localization of each protein. Interestingly, localization of AZ2 was shifted from the nucleus or nucleolus to the cytoplasm by c-MYC knockdown. We further investigated whether AZ2 accelerates c-MYC degradation in the nucleolus. Nucleophosmin 1, which is important for maintaining the nucleolus localization of c-MYC, was overexpressed in the cells, and then c-MYC degradation was monitored with overexpression or downregulation of AZ2. Overexpression of AZ2 increased degradation of endogenous c-MYC, whereas downregulation of AZ2 suppressed it. These results indicate the possibility that AZ2 accelerates the degradation of c-MYC in the nucleolus.

Analysis of interaction between AZ2 and ATP citrate lyase

ATP citrate lyase (ACLY) generates acetyl-CoA from mitochondria-derived citrate and is important for fatty acid synthesis and histone acylation. We have identified ACLY as an AZ2-interacting protein. To confirm a direct interaction of these proteins, we performed an *in vitro* pull-down assay using purified hemagglutinin-tagged ACLY, AZ1, and AZ2. Interestingly, ACLY bound not only to AZ2 but also to AZ1. To investigate how AZs affect ACLY activity, we measured ACLY activity *in vitro* and *in vivo* in the presence or absence of AZs. In the presence of AZs, ACLY activity was nearly doubled, and knockdown of AZs caused a decrease in ACLY activity. Polyamines did not affect

ACLY activity *in vitro*. These results suggest that AZs directly bind to ACLY and regulate ACLY activity.
We are also developing a method for measuring ACLY activity with a mass spectrometer using stable isotope-labeled citrates as a substrate.

Detection of posttranslational modification by polyamine
Polyamines covalently bind to proteins at glutamine residues by a transglutaminase reaction. This posttranslational modification by polyamine, namely polyamination, may change the activity or property of the protein. Little is known about target proteins and the physiological significance of the polyamination. We developed methods to detect polyamination. With putrescine-bound dimethyl-casein prepared by transglutaminase reaction as a sample, the molecular-related ion of gamma-glutamylputrescine was accurately detected with liquid chromatography mass spectrometry and a multimode octadecylsilyl column. Furthermore, the increased molecular mass by polyaminations at the 2 glutamine residues in a peptide fragment was detected by liquid chromatography tandem mass spectrometry analysis with low-flow captive spray ionization.
Mass-based global analyses of polyamination during changes in cellular polyamines and quantitative proteomics using stable isotope labeling will provide clues to the physiological significance of polyamination.

Multiple forms of mouse Azin1 messenger RNA differentially regulated by polyamines
We have found multiple forms of *Azin1* transcripts formed by alternative splicing and initiation of transcription from putative alternative start sites. This year, we first showed that stability of *Azin1* messenger (m) RNA was largely consistent in the presence or absence of 2-difluoromethylornithine, an inhibitor of polyamine synthesis. This finding indicates that polyamine does not affect the stability of *Azin1* mRNA. Next, to evaluate the physiological importance of Azin1, we examined the cell growth, in wild-type mouse embryonic fibroblasts ($MEFs^{+/+}$), and mutant MEFs ($MEFs^{-/-}$) in which expression of Azin1 is largely diminished. We found that cell growth rate of the mutant MEFs was increased by polyamine and by thymidine. We are now comparing expressed protein and metabolite profiling in both MEFs.

Isolation and analyses of polyamine-binding RNA aptamers
RNA aptamers have the potential for both clinical and research applications. In particular, 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 proposed a model of binding between spermine and a previously isolated anti-spermine aptamer. In this model 2 separated stem regions form 1 spermine-binding site with spermine-induced conformational change putting the 2 stems closer. This year, we performed stoichiometric analyses using isothermal titration calorimetry or a quartz crystal microbalance. From isothermal titration calorimetry, the specific 1:1 interaction between the aptamer and spermine was detected. With this assay, the dissociation constant (*K*d) was estimated to be 250 μM. With the quartz crystal microbalance assay, the specific inter-

action was also detected. However, the detected interaction value was much greater than expected. This greater value may come from some conformational change of the RNA aptamer induced by its interaction with spermine. These results support the binding model of this aptamer and suggest that spermine can induce conformational changes of RNA molecules that are the major binding partner for polyamines.

Publications

Matoba K, Kawanami D, Okada R, Tsukamoto M, Kinoshita J, Ito T, Ishizawa S, Kanazawa Y, Yokota T, Murai N, Matsufuji S, Takahashi-Fujigasaki J, Utsunomiya K. Rho-kinase inhibition prevents the progression of diabetic nephropathy by downregulating hypoxia-inducible factor 1α. *Kidney Int.* 2013; **84:** 545-54.

Murakami Y, Ohkido M, Takizawa H, Murai N, Matsufuji S. Multiple forms of mouse antizyme inhibitor 1 mRNA differentially regulated by polyamines. *Amino Acids.* 2014; **46:** 575-83.