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 to nucleic acids and are essential for proliferation. Cellular polyamine contents are maintained by a feedback mechanism involving the key regulatory proteins antizymes (AZs). AZs are expressed by translational frameshifting, which is induced by polyamines, and negatively regulate cellular polyamines. In mammals there are 3 AZ isoforms (AZ1–3). AZs are further regulated by proteins termed AZ 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 and diagnostic tools.

Research Activities

Role of AZ2 in c-Myc degradation under hypoxic conditions
AZ binds to ornithine decarboxylase (ODC), a key enzyme for polyamine biosynthesis, to trigger degradation of ODC by the 26S proteasome. AZ1 reportedly interacts with several proteins other than ODC and accelerates their degradation. We have previously found in cultured cells that AZ2 accelerates c-Myc degradation by proteasomes and that this pathway is involved in c-Myc decay after UV irradiation. This year we showed via knockdown of AZ2 that the proteasomal degradation of c-Myc under hypoxic conditions is also mediated by AZ2. Hypoxia has been reported to cause both c-Myc degradation and an increase in cellular polyamines. Thus, our results suggest a novel pathway of hypoxia-induced c-Myc degradation mediated by polyamines and AZ2 in a ubiquitin-independent manner.

Fluorescent visualization of cancer cells by monitoring of cellular polyamines
We are developing a novel method to visualize cancer cells by combining the polyamine-dependent frameshift mechanism of AZ, an endogenous cellular polyamine sensor, and fluorescent protein techniques. This year we improved the reporter construct by using the entire protein coding region of human AZ1 messenger (m) RNA with the enhanced green fluorescent protein (EGFP) gene that is inserted immediately downstream of the pseudoknot structure. Cells transfected with the construct showed increases in both EGFP fluorescence and the frameshift product in response to the addition of polyamines to the culture medium.

The effect of polyamines on hematopoiesis in adult mice
We have previously shown in AZ1 knockout mouse embryos that elevated tissue poly-

amine levels disturb early stage hematopoietic cell differentiation in the liver, particularly production of the multipotent hematopoietic progenitor cells (MPPs). To determine whether polyamines also affect hematopoietic cell differentiation in the bone marrow of adult mice, we fed a high-polyamine diet to adult mice and analyzed bone marrow cells. We found that the high-polyamine diet decreased the number of MPPs in adult bone marrow. Polyamines accumulate in patients with renal failure, and 10% to 15% of patients with chronic renal failure have anemia that does not respond to treatment with erythropoietin. Thus, accumulation of polyamines might be a cause of erythropoietin-resistant anemia in renal failure.

Multiple forms of mouse Azin1 differentially regulated by polyamines

Homozygous *Azin1* gene trap mice show partial lethality with decreased tissue levels of putrescine, but low levels of *Azin1* mRNA and Azin1 protein are detected in their tissues, raising the possibility that alternative forms of *Azin1* mRNA are transcribed to skip the trapping insertion. Last year, we found various splicing forms of *Azin1* mRNA. This year, we found 5 new alternative transcriptional start sites (TSS1-5) downstream of the trapping insertion site. TSSs1-3 are located upstream of the authentic starting codon and would explain the presence of the full-length Azin1 in the mutant mice. TSS4 is located on intron 4, and the corresponding transcript encodes an N-terminal-truncated form of Azin1 (Azin1ΔN). We also identified a splice variant with an extended exon 7 to the 5' direction which encodes a C-terminal-truncated form by a premature termination codon (Azin1ΔC). Both Azin1ΔN and Azin1ΔC retained antizyme-binding activity. Interestingly, the levels of transcripts for the full-length Azin and Azin1ΔC were reciprocally regulated by polyamines. These results suggest that polyamine-regulated splicing regulates Azin1 function by producing an alternative form of Azin1.

Analyses of the spermine-binding site on RNA aptamer

The technique of SELEX (systematic evolution of ligands by exponential enrichment) has been used to isolate high-affinity oligoribonucleotides called aptamers from randomized RNA libraries. Selected 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. The antispermine aptamer is predicted to contain 2 stem-loop structures (5' stem-loop and 3' stem-loop). Mutational analyses revealed that the 3' stem-loop bound spermine more effectively than did the 5' stem-loop. We identified a bulged (unpaired) structure in the 3' stem-loop (C/ACA) important for spermine binding, because a mutant without the bulge had markedly reduced binding activity. Further mutation analyses revealed that the A-U base-pair neighboring the bulged structure was also important for spermine binding. Moreover, ^{1}H-NMR (nuclear magnetic resonance) spectrum analysis revealed the G-U wobble base-pair next to the A-U pair is also concerned with spermine binding. These results suggest that this stem and bulge region is a hot spot for spermine binding.

Analysis of molecular mechanism of carcinogenesis in ovarian clear cell carcinoma

Amplification of chromosome 17q21–24 has frequently been observed in ovarian clear cell carcinoma (CCC). However, the driver gene of the region has not been identified. Aberrant expression of microRNAs has been shown to be involved in oncogenesis. MicroRNA-21 (miR-21) encoded on 17q21–24 is a frequently overexpressed microRNA in many types of cancer. On the basis of the above, we hypothesized that miR-21 plays important roles in CCC oncogenesis through the regulation of PTEN (phosphatase and tensin homologue) expression. Analysis of clinical samples revealed overexpression of miR-21 and repression of PTEN in cases of CCC with amplification of 17q21–q24.