

ATM-DEPENDENT DNA-DAMAGE RESPONSE AND TELOMERE METABOLISM LINK INDUCTION OF PREMATURE SENESCENCE IN ZEBRAFISH

Shuji Kishi

Dana-Farber Cancer Institute and Harvard Medical School

ATM (Ataxia Telangiectasia Mutated), the gene product mutated in Ataxia Telangiectasia (A-T) plays a pivotal role in a network of signal transduction in responses to genotoxic DNA damage. To study the physiological and developmental functions of ATM in zebrafish, we first obtained the full-length zebrafish ATM (zATM) cDNA, and analyzed the expression pattern of zATM during early development. Sequence analysis confirmed that zATM has high homology in the functional domains with both the human ATM and the *Xenopus* ATM. The FAT, phosphoinositide 3-kinase-like domain, and FATC domains, which regulate ATM kinase activity and functions, were the most highly conserved regions. ATM mRNA was detected by *in situ* hybridization in developing somites and central nervous system. We next demonstrated the effect of inhibition of ATM expression and function on embryonic development using antisense-morpholino oligonucleotides in zebrafish embryos. *In vivo* functional studies have shown that the knockdown of zATM causes an abnormal phenotype during development upon ionizing radiation (IR)-induced DNA damage, and also causes premature senescence even without DNA damage. In an attempt to obtain genetic mutations in zATM, we have also identified over 20 families of IR-sensitive zebrafish mutants induced by chemical mutagenesis using ethylnitrosourea (ENU).

On the other hand, ATM functions in telomere metabolism, and telomere maintenance and integrity play an important role in triggering of cellular senescence and subsequently the organismal aging process. Previously, we found that zebrafish have constitutively high telomerase activity in somatic cells throughout their lives, from embryos to adults. This is apparently different from mammalian telomerase regulation since in most mammalian somatic tissues telomerase activity is almost shut off or dramatically decreased in the early stages of development except for germ or stem cells. To elucidate the function of zebrafish telomerase during development and regeneration, we downregulated the enzymatic activity using antisense-telomerase inhibitors, targeting against zebrafish telomerase RNA component. Upon telomerase inhibition, we analyzed cell proliferation, apoptotic cell death, and cellular senescence *in vivo*. We found that zebrafish embryos with reduced telomerase activity displayed developmental retardation, telomere shortening and premature senescence. Adult fish intraperitoneally injected with the inhibitors exhibited a dramatic response in their regenerating caudal fin showing a decline in regenerative ability and an increase of senescence- β -gal activity. These results suggest that, in contrast to mammalian telomerase situation, zebrafish telomerase may become a rate-limiting factor for the prevention of senescence. Moreover the zebrafish's constitutive telomerase activity may be essential for the maintenance of homeostasis including their remarkable regenerative capacity.