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Molecular cloning and functional characterization of zebrafish ATM☆

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Abstract

Ataxia-telangiectasia mutated (ATM) is the gene product mutated in ataxia-telangiectasia (A-T), which is an autosomal recessive disorder with symptoms including neurodegeneration, cancer predisposition and premature aging. ATM is thought to play a pivotal role in signal transduction in response to genotoxic DNA damage. To study the physiological and developmental functions of ATM using the zebrafish model system, we cloned the zebrafish homolog cDNA of human ATM (hATM), zebrafish ATM (zATM), analyzed the expression pattern of zATM during early development, and further developed the system to study loss of zATM function in zebrafish embryos. Employing information available from the zebrafish genomic database, we utilized a PCR-based approach to isolate zATM cDNA clones. Sequence analysis of zATM showed a high level homology in the functional domains of hATM. The putative FAT, phosphoinositide 3-kinase-like, and FATC domains of zATM, which regulate ATM kinase activity and functions, were the most highly conserved regions, exhibiting 64–94% amino acid identity to the corresponding domains in hATM, while exhibiting approximately 50% amino acid identity outside these domains. The zATM gene is expected to consist of 62 coding exons, and we have identified at least 55 exons encompassing more than 100 kb of nucleotide sequence, which encodes about 9 kb of cDNA. By in situ hybridization, zATM mRNA was detected ubiquitously with a dramatic increase at the 18-somite stage, then more specifically in the eye, brain, trunk, and tail at later stages. To inhibit zATM expression and function, we designed and synthesized splice-blocking antisense-morpholino oligonucleotides targeting the phosphoinositide 3-kinase-like domain. We demonstrated that this knockdown of zATM caused abnormal development upon ionizing radiation-induced DNA damage. Our data suggest that the ATM gene is structurally and functionally conserved in vertebrates from zebrafish to human.

Keywords: A-T; ATM; Zebrafish; DNA damage; Aging

Abbreviations: A-T, ataxia-telangiectasia; ATM, ataxia-telangiectasia mutated; MO, antisense-morpholino oligonucleotide; PI3-K, phosphoinositide 3-kinase; FAT, FRAP, ATM and TRRAP related; FATC, FRAP, ATM and TRRAP C-terminal related; CNS, central nervous system; IR, ionizing radiation

☆ The nucleotide sequence reported in this paper has been submitted to the DDBJ/EMBL/GenBank database with accession number AB191208.

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1. Introduction

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder characterized by progressive cerebellar degeneration, immunodeficiency, cancer predisposition, gonadal atrophy, growth retardation, premature aging, and hypersensitivity to ionizing radiation (IR) (Lavin & Shiloh, 1997; McKinnon, 2004). Cells from A-T patients generally have short telomeres, and are also highly sensitive to IR (Kishi & Lu, 2002; Metcalfe et al., 1996; Meyn, 1995; Pandita, Pathak, & Geard, 1995; Shiloh, 1995; Smilenov et al., 1997). The molecular cloning of the gene responsible for A-T, ataxia-telangiectasia mutated (ATM), has allowed a better understanding of both ATM function and the A-T pleiotropic phenotypes (Savitsky et al., 1995a, 1995b; Taylor, 1998). The ATM gene encodes a nuclear phosphoprotein (Chen & Lee, 1996; Scott et al., 1998), with serine/threonine protein kinase activity for which many downstream molecules, such as p53, Chk2, Mdm2, NBS1, BRCA1, 53BP1 Smc1, FANC2, H2AX and Pin2/TRF1, which control cell cycle check points, DNA double-strand break or repair pathway, and telomere metabolism, have been identified as substrates (Kastan & Lim, 2000; Kishi & Lu, 2002; Kishi et al., 2001; Pandita, 2002; Shiloh, 2003). ATM functions as a potent protein kinase that is activated by DNA damage, such as IR, to phosphorylate target substrates. The identification of these potential substrates places ATM in a signal transduction pathway, through which it functions to regulate cell-cycle checkpoints that mediate the DNA damage response.

As a model system, ATM-knockout mice have been created in several laboratories by specific germline inactivation of the ATM gene (Herzog, Chong, Kapsetaki, Morgan, & McKinnon, 1998; Xu et al., 1996). Fibroblasts isolated from ATM-knockout mice display similar cellular phenotypes to those observed in cells from A-T patients (Elson et al., 1996). Also, phenotypically, ATM deficient mice display a variety of growth defects, meiotic defects, immunological abnormalities, radiation hypersensitivity and cancer predisposition, similar to those seen in A-T patients, confirming the most common pleiotropic roles of ATM. Early resistance to apoptosis in the developing central nervous system (CNS) of ATM-knockout mouse was observed after IR, especially in diverse regions of the CNS including the cerebellum (Herzog et al., 1998), which is markedly affected in A-T. Interestingly, the neurological defects in A-T become apparent early in life suggesting that they may likewise originate during development (Herzog et al., 1998).

ATM mRNA is expressed ubiquitously including the nervous system during embryonic development in mice (Chen & Lee, 1996). ATM protein has been detected in the nuclei of developing somites and the central nervous system in *Xenopus* embryo (Hensey, Robertson, & Gautier, 2000). ATM protein has also been found to localize in cytoplasm of neurons in adult mouse and human cerebellum (Allen et al., 2001; Barlow et al., 2000). ATM mRNA has been found to be ubiquitously expressed in mouse embryos with elevated mRNA expression levels in the cerebellum and other regions of the CNS, suggesting an early developmental requirement for ATM in the nervous system (Chen & Lee, 1996; Soares, Morgan, & McKinnon, 1998). However, little is known about ATM expression during early development in lower vertebrates, such as zebrafish.

Zebrafish has been recognized as a powerful model for genetic studies in development biology. Recently, the zebrafish system has provided insight into various human diseases such as neurodegenerative, hematopoietic, and cardiovascular diseases, as well as cancer (Amatruda, Shepard, Stern, & Zon, 2002; Grunwald & Eisen, 2002; Stern & Zon, 2003; Zon, 1999). As such, we decided to use zebrafish as a model system to study both the biological and the biochemical roles of ATM during development. The zebrafish model offers several unique advantages, such as large clutch sizes in reproduction, and rapid, external embryonic development, which allows direct visualization of organogenesis. Marked similarities between humans and zebrafish at the level of genes and genomic synteny have been well documented, along with conservation of developmental and signal transduction pathways (Bennett et al., 2001; Brownlie et al., 1998; Langenau et al., 2003; Liu et al., 2003). These attributes make the zebrafish a useful vertebrate model to investigate molecular pathways in human disorders, especially those affecting early embryologic development.

To develop a zebrafish A-T model, we first isolated zebrafish ATM (zATM) cDNA. Here, we provide zATM cDNA sequence as a predicted primary structure. We also outlined zATM expression patterns during early development of zebrafish embryos, and