Our Research Contributions in Epigenetics and RNA Helicases

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Abstract: We characterized the functions of previously uncharacterized human protein, DDX49 and reported that DDX49 is a RNA helicase and an important regulator of global protein biosynthesis. We reported that elevated levels of the well-studied RNA helicase, DDX39B contribute to oncogenesis by enhancing the stability as well as the synthesis of pre-ribosomal RNA and global protein synthesis. We recently reported that SET7/9 introduces methylation modification on eL42 and positively regulates the global protein synthesis. Mutations in the Urea cycle enzyme, ASS1 are associated with type I Citrullinemia disorder. Our group discovered that the ASS1 interacts with the PRMT7 enzyme and the ASS1 mutations associated with type I citrullinemia disrupt the interaction of ASS1 with PRMT7.

1 Introduction

I completed my Ph.D in 2009 with special distinction from the Jacobs University, Germany under the guidance of Prof. Dr. Albert Jeltsch. In Jacobs University, I contributed for developing a generalized method to map the interaction sites of protein complexes. During postdoctoral studies at Jacobs University, I was involved in the identification of several new non-histone target substrates for the protein lysine methyltransferase, SET7/9 and identified the effector function for the PWWP domain of DNA methyltransferase, DNMT3A. Subsequently, I joined Pondicherry University as Assistant Professor in the year 2010. In Pondicherry University, I am

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working in two major areas viz. (i) Histone arginine methylation mediated epigenetic signaling and (ii) Functional characterization of DExD box family of RNA helicases.

2 Research contributions from Jacobs University, Germany

SET7/9 and G9a are human protein lysine methyltransferases that are mis-regulated in several cancers and these enzymes are emerging therapeutic targets for cancers. But the molecular mechanisms underlying the pathological roles of these enzymes are not clearly understood. In this regard, during my research tenure at Jacobs University, Germany, I was involved in identifying several non-histone protein substrates of human SET7/9 and G9a. These findings provided new mechanistic insights into their functions [1,2].

In mammals, DNA methylation patterns are set by de nova DNA methyltransferase enzymes, DNMT3A and DNMT3B in co-operation with DNMT3L protein during embryogenesis [3,4]. We discovered that the PWWP domain of DNMT3A recognizes the trimethylation modification of lysine 36 of histone 3 (H3K36me3). Recognition of H3K36me3 modification by DNMT3A is required for the proper subnuclear localization of DNMT3A. This discovery propelled a lot of studies delineating the new roles of H3K36me3 modification [5].

Mutations in ATRX protein leads to alpha-thalassemia and mental retardation X-linked syndrome (ATR-X) [6,7]. We discovered that ADD domain of ATRX recognizes trimethylation modification of the histone 3 at K9 position (H3K9me3) and the mutations of ADD domain of ATRX that are linked to ATR-X syndrome disrupt the ATRX-H3K9me3 interaction. Our findings provided the molecular basis for the pathophysiology of the ATR-X mutations in the ADD domain [8].

3 Research contributions from Pondicherry University, India

As an independent scientist at Pondicherry University, my efforts are aimed to understand the functions of DEAD box family of RNA helicases and protein arginine methyltransferase enzymes. The significant research contributions are listed below.

3.1 Protein arginine methyltransferase 7 interacts with the urea cycle enzyme, argininosuccinate synthetase

Protein arginine methyltransferase 7 (PRMT7) generates mono methylation at the arginine residues of its protein substrates [9,10]. PRMT7 prefers to methylate the arginine residues in the sequence context of RXR motif in the basic amino acids rich regions [11]. We conducted yeast two-hybrid screening by using PRMT7 as a bait to find new interacting proteins for PRMT7. The yeast two-hybrid screening revealed that argininosuccinate synthetase (ASS1) as a putative interacting protein of PRMT7. ASS1 catalyzes the synthesis of argininosuccinate, a key rate limiting step of urea cycle [12]. Mutations that affect the functions of ASS1 leads to a rare autosomal disorder, type 1 citrullinemia [13,14].

We confirmed the PRMT7-ASS1 interaction in HEK293 cells through co-immunoprecipitation studies by overexpressing PRMT7 and ASS1 enzymes. Our immunoprecipitation studies revealed that endogenous levels of PRMT7 could interact with endogenous levels of ASS1. To study the nature of this interaction, we conducted GST and Ni-NTA pull down experiments with the recombinant PRMT7 and ASS1 enzymes and we observed the direct interaction of PRMT7 and ASS1. Besides, our co-localization studies also supported the in vivo interaction of PRMT7 with ASS1.

We predicted the interface of PRMT7-ASS1 complex by using computational methods and substantiated the predicted interface through co-immunoprecipitation and GST pull down experiments using mutant ASS1 proteins. Our evolutionary analysis indicated that the residues of ASS1 which are involved in the interaction are less conserved in the organisms that lack PRMT7 and strongly conserved in the organisms that contain PRMT7 indicating that they have co-evolved with PRMT7. We observed that the citrullinemia mutations of ASS1 that are located at the interface of PRMT7-ASS1 complex disrupt the interaction of ASS1 with PRMT7. This work was published in Journal of Molecular Biology [15].

3.2 Functional characterization of the RNA helicase, DDX49

DDX49 was an uncharacterized protein and it is grouped in the DExD helicase family based on the conservation of sequence motifs. It was predicted that DDX49 might possess a RNA helicase activity [16]. We reported that DDX49 shows a strong helicase activity on RNA and it is much stronger than the RNA helicase activity of DDX39B, a well characterized protein with important biological functions and a homolog of DDX49 [1722]. Since some members of the DExD helicase family are implicated in mRNA export from nucleus to cytoplasm [17,2325], we studied the role of DDX49 on the export of mRNA using fluorescence in situ hybridization method

and quantifying the cytoplasmic mRNAs through quantitative RT-PCR. We found that DDX49 is needed for the export of mRNAs from the nucleus to cytoplasm.

Further, we investigated the sub-cellular localization of DDX49 and found that DDX49 was localized predominantly in the nucleolus. Hence, we investigated the function of DDX49 on the regulation pre-ribosomal RNAs and found that DDX49 is essential to maintain the levels of 47S pre-ribosomal RNAs. Our mechanistic studies revealed that DDX49 regulates the pre ribosomal RNA levels by regulating its synthesis and stability. Chromatin immunoprecipitation studies indicated that DDX49 is associated with the promoter regions of rDNA locus. Since pre-ribosomal RNA levels are regulated by DDX49, we studied the role of DDX49 on global protein synthesis through puromycin assay. We observed that DDX49 is essential for the efficient global translation. Since the DDX49 regulates the global protein synthesis, we studied the role of DDX49 on cell proliferation and clonogenic capacity of the cells. We found that over-expression of DDX49 increased the cell proliferation and clonogenic capacity of the cells suggesting that increased levels of DDX49 might contribute for the oncogenic transformation of the cells. In fact, analysis of DDX49 expression levels in BioXpress database revealed that DDX49 levels are elevated in several cancers of different types. This whole work was published in Nucleic Acids Research [26].

3.3 DDX39B enhances global protein synthesis

The RNA helicase, DDX39B belongs to the DExD box helicase family and well-studied member. DDX39B is implicated in RNA splicing and the export of poly(A)+ RNAs from nucleus to cytoplasm [17,22,27,28]. Since it is reported that elevated expression of DDX39B increases the global protein synthesis [29], we investigated that function of DDX39B on the maintenance of pre-ribosomal 47S RNA levels. We discovered that the DDX39B regulates pre-ribosomal RNA levels and the over-expression of DDX39B increases its levels by more than three folds. Our actinomycin D assay, 5-fluorouridine incorporation assay and chromatin immunoprecipitation analysis revealed that DDX39B promotes the transcription and the stability of 47S ribosomal RNAs. Since DDX39B regulates the 47S ribosomal RNA levels, we analyzed the role of DDX39B on global protein synthesis, cell proliferation and clonogenic capacity of the cells. We found that DDX39B enhances the global translation which in turn enhances the cell proliferation and clonogenic capacity of cells indicating the oncogenic potential of DDX39B. Analysis of DDX39B expression levels in BioXpress database indicated that DDX39B levels are increased in several types of cancers. This work was published in RNA Biology [30].

3.4 SET7/9 methylates eL42 and regulates translation

The protein lysine methyltransferase, SET7/9 methylates wide variety of substrate proteins and regulates numerous biological processes [31]. We recently identified the ribosomal protein, eL42 as an interaction partner for SET7/9 through yeast two-hybrid screening and interaction was validated in mammalian cells by using co-immunoprecipitation studies. Our GST pull down analysis revealed that the MORN domain of SET7/9 directly interacts with eL42. We found that SET7/9 methylates eL42 at three different lysines. Our puromycin assay indicated that methylation of eL42 by SET7/9 positively regulates the global protein synthesis. This study identified a new cellular process that is regulated by SET7/9. This work was published in BBA-Molecular Cell Research [32].

4 Summary

Our research findings on the RNA helicases, DDX49 and DDX39B deepens our understanding on the molecular functions of these enzymes and shed light on their potential oncogenic functions. The finding that the SET7/9 introduces methylation modification on eL42 and this methylation event regulates the protein synthesis adds a new regulatory role to the SET7/9 enzyme.

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