RB20 is a RNA-binding protein that acts as a regulator of mRNA splicing of a subset of genes that play a key role in cardiac function [1, 2]. Recent studies have indicated that mutations in RB20 are associated to human dilated cardiomyopathy [3]. Most of the point mutations are clustered in exon 9 of the gene, which encodes an RS-rich domain. RS domain of SR-proteins can interact with both RNAs and proteins, can confer shuttling property or acts as Nuclear Localization Signal. Mutations in this domain can impair at multiple steps the post transcriptional regulation associated to cardiovascular pathologies.

**Fig. 1:** Loss-of-function mutation in the splicing factor RB20: normal and aberrant splicing of cardiac proteins (Linke et al., Nat Med. 2012).

**SCOPE**

The functional motifs of the RB20 protein have been poorly investigated. Our study aims to analyze the protein domains required for the nuclear localization of RB20.

**MATERIAL AND METHODS**

**Bioinformatics analyses.** The RB20 protein sequence (G5T4B1) was investigated for the presence of canonical NLSs using PSORT II server and by the predictNLS program.

**RT-PCR.** RNA was extracted by human and mouse tissues with Trizol reagent and retrotranscribed with Superscript III (Invitrogen).

**Construction of expression vector.** Total RNA was isolated from cardiac human tissue and C2C12 mouse cells. Fragments were amplified and cloned into the Vivid Colors™ N-EmGFP TOPo® Expression Vector Kits (Invitrogen). A series of truncated peptides of human and mouse RB20 fused to GFP was created by PCR from the cDNA containing the open reading frame. Plasmid DNAs were transiently transfected into HeLa cells. After 24h, the direct fluorescence of GFP-RB20 fusion proteins and the immunofluorescence of others splicing factors were analyzed by fluorescence confocal microscopy.

**RESULTS**

1. **Molecular organization of human RB20.** The human RB20 gene is located on 10q25.2 chromosomal region and organized in 14 exons; it encodes a protein of 1227 aminoacids, which contains an RNA recognition motif (RRM), an RS domain (RS) and a Zn²⁺ finger domain (ZF).

2. **Subcellular localization of RB20 protein.**

   Over-expression of RB20 in HeLa cells showed a nuclear localization with nucleolar exclusion and punctuated distribution that resembles the nuclear speckles typical of SR proteins (Fig. 2). No co-localization with splicing factor SC35 was seen and only a minor fractions of PTB speckles were merged with RB20.

**Fig. 2:** Nuclear distribution of RB20, SC35 and PTB in HeLa RB20 transfected cells. Scale bar: 5µm

3. **Functional domains required for subcellular localization.** Vectors expressing GFP fused to human (HRBM20) or mouse (mRBm20) protein were utilized to analyze the contribution of the protein domains to nuclear localization.

**Fig. 3:** A Functional domains of human (left) and mouse (right) RB20 protein. B Subcellular localization of wild-type and mutant RB20 proteins.

Deletion of RRM and RS domains impaired the exclusive nuclear localization (658-1227 construct). C-ter and N-ter of the protein do not contribute to localization of RB20 as seen in 1-658 and 486-1199 constructs, respectively (Fig. 3).

4. **RB20 contains nuclear retention determinant.** Exclusive nuclear localization was observed in all transfected cells with constructs that retained RRM, RS domains and the region between them.

**CONCLUSION**

In the present work we isolated and cloned the cardiac isoform of RB20 and shown that it is distributed exclusively into the nuclei of transfected cells. Our study provides functional annotations to structural domain within the RB20 protein required for its subcellular localization. They depicted it as a nuclear SR protein due to the presence of a Dominant Nuclear Retention Signal extended into RRM and RS domains encompassing an highly conserved stretch of basic aminoacids. This may help to better understanding the molecular mechanisms that are impaired in post transcriptional regulation associated to cardiovascular pathologies that involve the RB20 protein.

**REFERENCES:**

