THE REPRESSION OF NFAT1 TRANSCRIPTIONAL ACTIVITY MEDIATED BY IRF2BP2 PROTEIN INVOLVE SUMO PATHWAY

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The NFAT (Nuclear Factor of Activated T Cell) family of transcription factors regulates genes involved in various aspects of cellular function. The calcium regulated-NFAT members (NFAT1-4) and the different isoforms share high conservation. However, divergent functions between NFAT proteins in cell transformation may exist, but have not yet been characterized. Interestingly, our laboratory has recently identified a transcriptional repressor, IRF2BP2 (Interferon Regulatory Factor-2 Binding Protein 2), which specifically interacts with NFAT1 protein among the NFAT family members and represses its transcriptional activity. The aim of the present study is to elucidate the molecular basis of this repressive mechanism. Moreover, since the regulation of IRF2BP2 is not clear yet, another objective is analyzing post-transcriptional modifications as a possible regulating mechanism of this protein. We performed a two-hybrid screening using IRF2BP2 protein as a bait. The UBE2I SUMO-conjugating enzyme and SUMO1 (Small ubiquitin-like modifier) were identified as possible partners of IRF2BP2. Analysis to confirm interaction between IRF2BP2 and UBE2I were realized by yeast two-hybrid, co-immunoprecipitation, His-pull down and FRET. After that, luciferase-transactivation assays to observe the effect of SUMO pathway in repressor phenotype of NFAT1. Moreover, sumoylation assays were performed to investigate the SUMO-modification of IRF2BP2. Our analysis confirmed the interaction of IRF2BP2:UBE2I. Furthermore, analysis by confocal microscopy demonstrated that these proteins co-localized in the nucleus. Moreover, the interaction occurs independently of N-terminus C4-zinc finger of IRF2BP2 as shown in yeast two-hybrid interaction assays. And the luciferase-transactivation assays suggests that SUMO1 pathway decrease the repressor phenotype of NFAT1 mediated by IRF2BP2. On the other hand, sumoylation assays showed that three SUMO1 molecules are added to residues of IRF2BP2. Post-transcriptional modifications are important forms of protein activity regulation and show different biological outcomes. Our data suggest that IRF2BP2 is SUMO1-modified protein and SUMO pathway is involved in the repression of NFAT1 by IRF2BP2.

Financial support: CNPq, FAPERJ, ICGEB, INCT-Cancer.