Abstract

Poly(A)-specific ribonuclease (PARN) is a deadenylase, an effector of eukariotic mRNA decay that also mediates the maturation of a diverse and expanding repertoire of non-coding RNAs. Not unlike most protein coding genes, PARN encodes for multiple splice variants many, of which have been detected previously by RNA sequencing. Such splice variants have not been biochemically characterized or studied specifically in any context. In this work we detected the expression of a PARN splice variant in pleural malignant mesothelioma (PMM) cell lines and lung fibroblasts, the splice variant is co-expressed along with PARN in the PMM cell lines but not in lung fibroblasts. Our findings reveal that the splice variant is expressed at both the mRNA and protein level, in cell lines derived from all three pleural malignant mesothelioma subtypes, whilst being barely detectable in benign pleural immortalized cells, the highest expression levels were detected in lung fibroblasts. We identified and cloned its coding region and subsequently overexpressed, purified and biochemically characterized its protein product. Additionally the splice variant wa Cloning and sequence analysis revealed that compared to PARN mRNA it lacks 83 bases from the 3’ end of exon 1, this spliced out region includes the original start codon of PARN, leading PARN\_v1 to be translated through a downstream start codon located at exon 4. The resulting protein lacks a large 61aa region of the original PARN nuclease domain, including two of the main catalytic residues D28 and D30. Unexpectedly the novel protein was found to retain its ability to deadenylate possibly through the steric substitution of D28 and D30 by D292 and E378 respectively. To examine the biological role of PARN\_v1, it was silenced in lung fibroblasts and subjected to mass spectrometry, revealing its role in cell adhesion and cell-ECM interactions. Upon fibroblast differentiation to cancer associated fibroblasts by the incubation of fibroblasts with breast cancer cell cultured media and breast cancer cell derived exosomes, the PARN\_v1 levels serverely diminished and PARN expression was activated. Finally we developed an exosome isolation protocol for the quick detection of established exosomal RNA biomarkers in pleural fluid.