Identification, biochemical characterization and intracellular localization of sponge homolog of RRAS2

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Cancer is one of the most extensively studied diseases that occurs in almost all multicellular organisms. It is most likely that cancer appeared in parallel with multicellularity and the development of true tissues and organs. Thus, investigation of proteins involved in processes of intercellular cooperation, cell division control and multicellularity is crucial for better understanding of the disease. Comparative genomic analyses have shown that most genes/proteins associated with human cancer emerged during the early evolution of Metazoa. Hence, the study of these proteins in simpler organisms, such as sponges, provides a new approach in understanding cancer. Sponges are important model organisms because of their simple morphology and a complex genome with many genes/proteins highly similar to their vertebrate homologs. Using bioinformatics tools, we identified a homolog of human RRAS2 (*Ras-related protein R-Ras2*), a cancer-related protein, in the freshwater cave sponge *Eunapius subterraneus*. RRAS2 (also known as TC21) belongs to the subfamily of RAS-related proteins. When constitutively expressed and activated, it has a regulatory role in cell proliferation and migration, and is often overexpressed in human cancers. Our aim is to understand the physiological functions of RRAS2 protein in humans using sponges as a model system and to gain a better insight into the evolution of this oncogene. Bioinformatics analysis showed high conservation of RRAS2 protein and its homologs among Metazoa. The sponge cDNA encoding RRAS2 protein was successfully cloned into the expression vector and overexpressed. We confirmed GTPase activity of sponge RRAS2 protein. Additionally, cDNA was cloned into vectors for biological characterization of RRAS2. Intracellular localization of sponge RRAS2 protein was determined in transfected human tumour cells using immunofluorescence and confocal microscopy.