

DECIPHERING THE INTERACTOME OF p8, A PROTEIN RELATED TO TUMOR PROGRESSION

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p8 is an 8 kDa protein. It was identified due to its induction during the acute phase of pancreatitis. Functions related to cell growth control and stress have been attributed to p8 since its mRNA levels are increased in response to stress and mitogenic factors. An important role in tumor progression was also assigned to p8 since it was observed that p8 expression is altered in various malignant processes; and that while fibroblasts obtained from p8 *+/+* mice transformed with a retroviral vector expressing oncogene E1A are able to induce tumor formation when injected into nude mice, transformed fibroblasts derived from p8 *-/-* mice have no tumorigenic properties.

Analysis of its sequence identified a conserved region corresponding to a NLS. Immunocytochemistry experiments show that the sub-cellular localization of p8 depends on cell culture density, cell cycle and acetylation state of the cells. Its nuclear import is energy dependent, and the NLS of p8 is necessary and sufficient to retain a heterologous protein in the nucleus.

The fact that it is small enough to diffuse between nucleus and cytoplasm, but still possesses a NLS and a controlled localization suggests that it could associate to multiprotein complexes. Our aim is to identify these complexes. We generated a HEK293 cell line that expresses p8 fused to HIS-FLAG tags. Tandem affinity purification was performed. The purified complexes were digested and analyzed by LC-MSMS to identify proteins associated to p8.

We generated a list of proteins that interact with p8 and combined it with previous two-hybrid experiments and bibliographical data to generate the interactome, which shows that p8 associates to proteins related to DNA transcription, repair and mRNA processing. This supports the hypothesis that p8 is a multifunctional protein that interacts with different proteins in different cellular compartments to perform different functions. We cannot disregard the possibility that p8 could be mediating the import of protein complexes into the nucleus.