Universität Basel

Research Project

Characterization of a novel translation readthrough isoform of Argonaute 1 in human liver cancer

Third-party funded project

Project title Characterization of a novel translation readthrough isoform of Argonaute 1 in human liver cancer
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AGO1x is a novel, uncharacterized translational readthrough isoform of AGO1, an Argonaute protein with key roles in the post-transcriptional mechanisms of gene regulation known as RNA interference (RNAi). Hypothesizing, based on preliminary work, that AGO1x contributes to the growth of liver cancer cells through an uncharacterized, miRNA-independent pathway, in this project, I aim to uncover the function of AGO1x in liver cells. Specifically, I aim to characterize 1) the phenotypic effects and 2) the global molecular effects of AGO1x perturbation; as well as to 3) identify the direct targets of AGO1x. Towards these aims, I am currently generating AGO1x-knockout cell lines by disrupting the translation readthrough of AGO1 3'UTR through CRISPR/CAS9-mediated genome editing. I am generating mutants of two liver cancer cell lines with high AGO1x endogenous expression. I will then perform in vitro phenotypic (e.g. soft agar, migration and invasion assays, cell cycle analysis) and molecular assays (RNA sequencing and liquid chromatography-mass spectrometry (LC-MS)) on the parental and derivative cell lines to evaluate the role of AGO1x in liver cancer lines. The next objective is to identify the direct targets of AGO1x in liver cancer cells by pulling down and sequencing the RNA bound to the AGO1x protein using the Photoactivatable Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) method or comparable approaches. To increase specificity, I will intersect the results with those from PAR-CLIP in cell lines with ectopic expression of a tagged form of AGO1x. A long term goal is to test the hypothesis that AGO1x has a non-canonical role in modulating the levels of dsRNA species, which mimic viral dsRNAs and may thus trigger an innate immune response in liver cancer cells. I will evaluate the levels of dsRNAs with the dsRNA-specific J2 antibody in parental and AGO1x-knockout liver cancer cell lines and I will determine the origin of these dsRNAs with a modified CLIP protocol.ă

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Published results

4603336, Ghosh, Souvik; Guimaraes, Joao C.; Lanzafame, Manuela; Schmidt, Alexander; Syed, Afzal Pasha; Dimitriades, Beatrice; Börsch, Anastasiya; Ghosh, Shreemoyee; Mittal, Nitish; Montavon, Thomas;

Correia, Ana Luisa; Danner, Johannes; Meister, Gunter; Terracciano, Luigi M.; Pfeffer, Sébastien; Piscuoglio, Salvatore; Zavolan, Mihaela, Prevention of dsRNA-induced interferon signaling by AGO1x is linked to breast cancer cell proliferation, 0261-4189 ; 1460-2075, The EMBO Journal, Publication: JournalArticle (Originalarbeit in einer wissenschaftlichen Zeitschrift)

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