Aram Ghalali

Oncogener och läkemedelsutveckling för aggressiva former av cancer.

Validate AZIN1 as a target for medulloblastoma treatment. I obtained funding from The Sweden-America Foundation for a postdoc project. I have been investigating whether oncoprotein AZIN1 can be identified as a marker for medulloblastoma. So far, I have used tissue micro arrays (TMA) and have stained 38 cases of astrocytoma, 24 medulloblastoma (21 from TMA and 3 fresh tissues), 14 cases of glioblastoma, 6 cases of oligodendroglioma, 1 case of ependymoma, 3 cases of cancer adjacent brain tissue and 3 normal brain tissues.

I have seen massive upregulation of AZIN1 in all brain cancer tissues vs control normal or adjacent tissues. I have established a collaboration with the Children's Oncology Group (COG) *(see attached letter, approved protocol number: ACNS20B1-Q)* which is a National Cancer Institute (NCI) established cooperative group that includes over 8,000 pediatric cancer specialists located at approximately 200 medical centers (the COG Member Institutions) in the United States, Canada, Mexico, Australia, New Zeeland and selected countries in Europe. COG conducts clinical trials to establish improved treatments for children with cancer, and to translate new laboratory and clinical research findings to new therapies. The COG has 48 medulloblastoma cases that I will have access to. I will also stain those 48 cases for the AZIN1 expression and investigate possible association to disease features such as demographic, biologic, clinical, or outcome.

Lately, it has been shown that AZIN1 undergoes RNA editing which results in a serine-to-glycine substitution at residue 367. This editing event was shown to be important for AZIN1-induced tumor aggressiveness and shortened patient survival. I have now constructed 3 different plasmids, coding for different variants of AZIN1; a wild-type-AZIN1 which has AGC at the codon 367, an edited-AZIN1 (IGC at the codon 367) and a noveluneditable-AZIN1 in which the codon 367 is edited to TCC and therefore has no Adenosine residue and is thus unaffected by ADAR enzyme which is responsible for RNA editing in cancer cells. Using Gateway cloning, these inserts have been introduced in pCW57.1 The pCW57.1 is an ideal model since it contains an inducible promoter. This is primarily characterized by a strong and tight controllable regulation, a cost-efficient induction, and an effective expression of the gene of interest being placed downstream of the inducible promoter sequence.

Using HEK293T cells, lentivirus containing the plasmid of interests have been obtained. Three different human medulloblastoma cell lines; D283, D458, and D556 human medulloblastoma cells have been stably overexpressed for wtAZIN1, edAZIN1 and uneditable AZIN1. Optimizing the doxycycline concentration (the inducer) to obtain a physiological levels of AZIN1, I have obtained convincing data showing that AZIN1 overexpression in D283, D458, and D556 human medulloblastoma cells increases aggressiveness as measured by proliferation, invasion, and anchorage-independent growth.

Perhaps the most important finding is that we are the first to find that AZIN1 is secreted to the cerebrospinal fluid (CSF) in the M1 subtype of medulloblastoma (using both ELISA and Western Blotting). AZIN1 is also secreted in D283, D458, and D556 human medulloblastoma cells, but not the human fetal glial cell line SVG (a control cell line). Following up medulloblastoma patients, the AZIN1 level decreases after surgery and the cancerous tissue is removed. However, we have limited number of follow up patients but hope to increase these numbers in the coming year.

I have also transiently overexpressed an AZIN1-clover fused plasmid. This plasmid gives the opportunity to see where AZIN1 is localized since clover is the brightest known green fluorescent protein and it will now label AZIN1 *in vivo*. AZIN1 is localized in extracellular vesicles when AZIN-clover plasmid is overexpressed in medulloblastoma cells.

Medulloblastoma is traditionally subdivided based particular molecular features. The Myc amplification categorizes one such subgroup., In my studies, I find high AZIN1 secretion in patients with the Myc gene amplification. In collaboration with Researchers from the Wyss Institute at Harvard, performing the multivariable models, we have found strong correlation between Myc and AZIN1 in 223 medulloblastoma patients (p- value 4.88 e-09).

I am currently working to investigate whether AZIN1 activity/level is correlated to other genes/pathways that were previously shown to be important features for this disease (see below). Although nearly all medulloblastoma occurs sporadically, there are three known

inherited syndromes associated with medulloblastoma:

- Gorlin syndrome which is associated with mutation in the PTCH1, PTCH2 or SUFU genes resulting in deregulation of the Sonic Hedgehog (SHH) pathway;

- Turcot syndrome which is characterized by mutation in APC gene resulting in deregulation of the WNT/beta-catenin pathway.

- Li Fraumeni syndrome characterized by p53 mutations resulting in familial cancer predisposition.

My overall goal is to investigate whether AZIN1 offers a potential therapeutic target for medulloblastoma therapy. My findings are described in a manuscript in preparation, in which I am both **senior** and **corresponding** author. I am determined to submit it for publication during 2022.Thanks to your generous funding, I believe that I have made important contribution to the childhood brain cancer field, especially after my findings are published. I am sure that my recent findings and future discoveries will change the thinking in this field. Therefore, I am preparing my resume to be a competitive researcher in the field of pediatric oncology and will apply for 2023 call from the Swedish Childhood Cancer Fund for research projects relevant for pediatric oncology within the area of biomedical science (4 or 6 years call).

I have also studied the role of AZIN1 in aggressiveness of prostate cancer and that work has been recognized and by an American Association for Cancer Research-Prostate Cancer Foundation Scholar-in-Training Award (2020). At the annual meeting of the AACR, my was selected as “a meritorious proffered paper selected for presentation”, an honor accorded to only a small number of presentations.

As I have curried this research independently and have been able to establish fruitful collaborations and secure my own funding. My PIs and the Vascular Biology Program's Chairperson, Dr. Marsha Moses, has promoted me to the position of Instructor (faculty) at Harvard Medical School. All of these scientific and career advancements would have been considerably more difficult without the generous funding from the Sweden-America Foundation.