

# **Dysregulation of Arginase Isoenzymes in FL-HCC:** Investigating the Impact of Nonspecific Arginase-Isoform Antibodies on the Market

Hypothesis 1: In all FL-HCC tumor samples ARG2 expression is upregulated.

**Hypothesis 3:** There are commercially available arginase antibodies that are isoform specific.

Aims: To test the hypothesis by comparing the expression levels of the ARG1/ARG2 between individual patient liver tumor samples vs normal liver samples using western blot analysis while testing the isoform-specificity of commercially available antibodies

Schematic of the Molecular Cause of FL-HCC

of DNA between two genes, creates the fused DNAJB1-PRKACA gene called a chimera.

Α	DN	AJB1	(~400kb)	PRKACA					
	Normal Chromosome 19								
В	Exon 1	Deleted		Deleted	Exon 2-10				
		DNAJB1	PRK	ACA					
	FLHCC Chromosome 19								





What is arginase Arginase is an enzyme and cell proliferation. S arginine to ornithine ar	<b>?</b> That plays an import Specifically, arginase nd urea.	imonia Arginine $Arginase$ Ori on of $H_2O$ Urea	nithin	
	Isoform	ARG1	ARG2	
	Size	35 KDa	40 KDa	
Prin	Primary Location Liver Kidney		Kidney	
Ir	ntracellular Location	Cytosol	Mitochondria and Cytosol	
	Function	Hydrolyze arginine in the cytosol	Hydrolyze arginine in the cytosol and mitochondria	

in the cytosol and the other pathway takes place in the mitochondria.



Sofia Dodge, Allysa Steinhaus, James Dinger, Riki Mae Duevel Faculty Advisor: Dr. Mary Ann Yang, Concordia University St. Paul

obtained in 2023.

### **Isoform Specificity of ThermoFisher ARG2 Antibody**



## • [Conclusion 1] ARG2 is up-regulated in FL-HCC samples

- [Conclusion 2] ARG2 TF (MA5-36204) antibody is isotype specific
- [Conclusion 3] ARG1 is stage or tumor specific in FL-HCC samples normal liver samples

- According to UniProt, ARG2 is strongly expressed in the kidneys and prostate, less strongly expressed in the brain, skeletal muscles, placenta, lungs, mammary gland, macrophage, uterus, testis and gut. However ARG2 is NOT expressed in the liver, heart or pancreas.
- ARG2 is expressed in liver tumors.

- It was found that many commercially sold arginase antibodies that are marketed as isoform specific are actually not isoform specific.
- Although antibody companies are able to target a specific isoform, most neglect to verify that the antibody does not detect other isoforms. • Isoforms have very similar amino acid sequences
- which makes is difficult to target a specific isoform. • Cross-reactivity occurs due to the overlapping
- sequences of ARG1 and ARG2.

### [Discussion 3] Significance of ARG1 showing stage specificity in FL-HCC samples • We hypothesized that upregulation of ARG1 in earlier stages of FLHCC might serve to help cell proliferation and in

turn promote tumor growth.

### [Discussion 4] Increased expression of arginase plays a role in tumorigenesis

- Intracellularly, increased arginase in tumor cells can lead to increased cell proliferation.
- Extracellularly, decreased free arginine in the tumor microenvironment results in decreased T-cell proliferation and decreased T-cell activation.
- Decreased free arginine in the tumor microenvironment is the result of increased use of arginine in tumor cells and the sequester of extracellular arginine.
- The increased use of arginine in tumor cells leads to decreased cancer immunosurveillance.

# [Discussion 5] Arginase as a potential druggable target

- upregulation of arginase.
- line)
- of creating an isoform specific arginase inhibitor.

ARG1; [Hek]: Human Embryonic Kidney Cell-line References

Tissue expression of Arg2 - staining in liver - the human protein Atlas

National Center for Biotechnology Informatio

Uniprot website . UniProt



• ThermoFisher (TF) ARG2 antibody targets the C-terminus, which contains differences in the amino acid sequences.

• ThermoFisher ARG2 antibody did not detect purified ARG1 protein; demonstrating isoenzyme specificity.

### Conclusions

• FL-HCC samples show up-regulation of ARG2 in comparison to normal liver samples.

• FL-HCC samples show dysregulation of ARG1 that can either be up-regulated or down-regulated in comparison to

### Discussion

### [Discussion 1] Significance of ARG2 showing up-regulation in FL-HCC samples





HCC

Arg2

Fig. 7. IHC staining of ARG2 in a liver tissue sample from (A) a 32 year old female with a healthy liver (B) a 38 year old male with metastatic liver adenocarcinoma.

### [Discussion 2] Significance of ThermoFisher ARG2 antibody being isoform specific

Fig. 8. Western blot analysis of arginase isoenzyme ARG2 in control liver and in hepatocellular carcinoma. Lines 1 and 6-standard human kidney ARG2; lines 2, 3-control liver; lines 4,5-HCC.(Chrzanowska et al. 2008)

HCC

• Using the correct negative control is important to verify the isoform specificity of an antibody.

• Non-isoform specific antibodies being sold as isoform specific reduces the validity of published research.

Chrzanowska et al. likely used an ARG2 antibody that was not isoform specific.

• The use of ARG2 works as a positive control. But without using ARG1 as a negative control, *Chrzanowska et al.* were unable to be certain that they were only targeting ARG2.

• There should not be ARG2 expression in the liver samples.

• In later stages of FL-HCC, the cells are no longer performing regular liver cell functions such as detoxifications which requires arginase. ARG1 is then downregulated to focus energy on other tumor specific functions.

• *Clemente et al.* noted that a majority of pathologies related to dysregulation of arginine are connected to the

• nor-NOHA, BEC and ABH are commercially available competitive arginase inhibitors. These commercially available arginase inhibitors have been clinically tested on cancers such as colorectal, breast, lung, liver etc. • Niu et al. reported that nor-NOHA induced cell apoptosis and inhibited invasion and migration of HepG2 (HCC cell

### • Currently, there are no isoform selective arginase inhibitors on the market.

• The structural differences between the active sites of ARG1 and ARG2 are very minimal, this increases the challenge

### Legend/References

Legend: [ARG1 (Pro)]: Proteintech Brand Catalog#: 66129-1-lg; [ARG2 (IN)]: Thermo Fisher Catalog#: PA527987; [ARG2 (Bio)]: Biotechne Brand Catalog#: MAB10602; [ARG2 (TF)]: Invitrogen Brand Catalog#: MA5-36204; [PD-ARG1]: Purified

Chrzanowska, A., Krawczyk, M. & Barańczyk-Kuźma, A. Changes in arginase isoenzymes pattern in human hepatocellular carcinoma. Biochemical and Biophysical Research Communications 377, 337–340 (2008 Matos, A., Carvalho, M., Bicho, M. & Ribeiro, R. Arginine and arginases modulate metabolism, tumor microenvironment and prostate cancer progression. Nutrients 13, 4503 (2021).

Niu, F. et al. Arginase: An emerging and promising therapeutic target for cancer treatment. Biomedicine & Pharmacotherapy 149, 112840 (2022). S. Clemente, G., van Waarde, A., F. Antunes, I., Dömling, A. & H. Elsinga, P. Arginase as a potential biomarker of disease progression: A molecular imaging perspective. International Journal of Molecular Sciences 21, 5291 (2020

You, J. et al. The oncogenic role of ARG1 in progression and metastasis of hepatocellular carcinoma. BioMed Research International 2018, 1–10 (2018)

