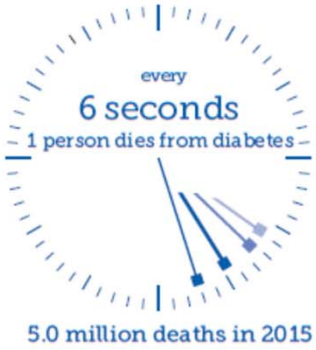
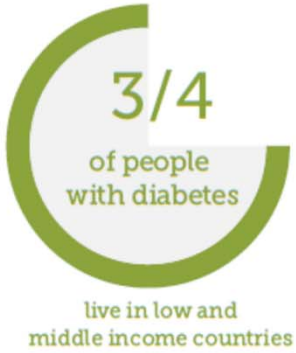
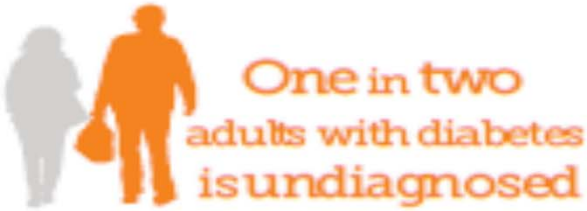
Long non-coding RNA HOTAIR –a therapeutic target and a biomarker (with other lncRNAs) for diabetic retinopathy

Diabetes: Global perspective







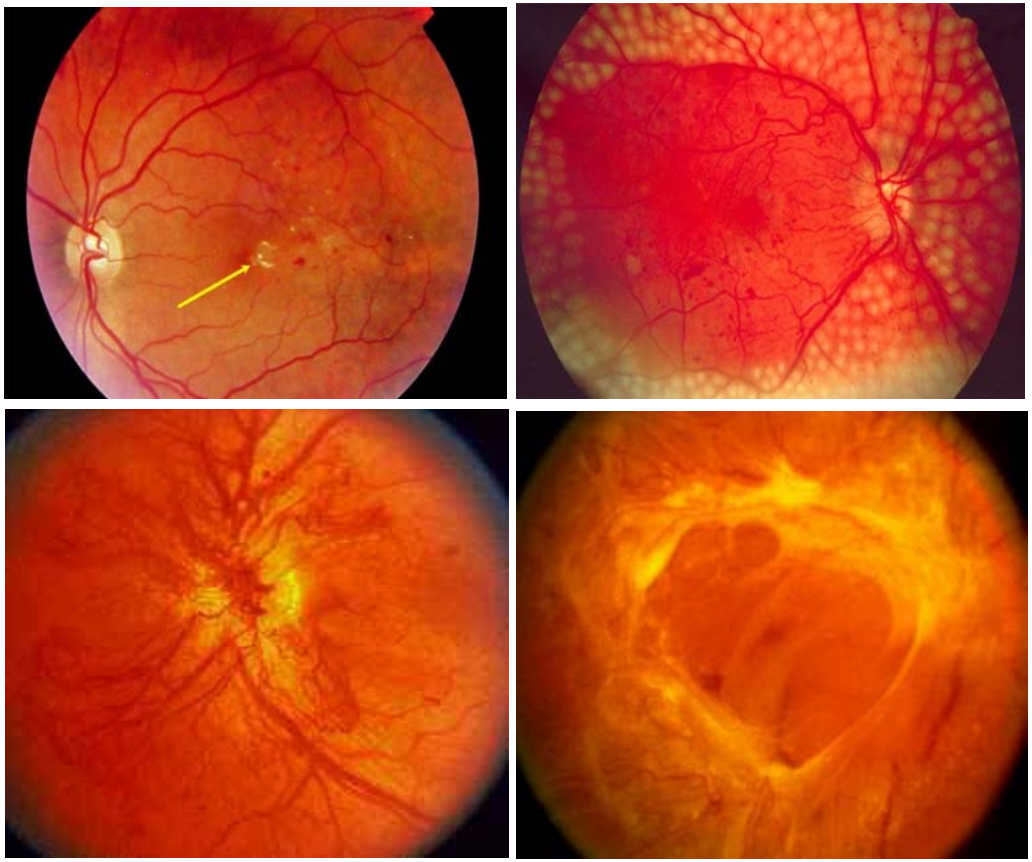
IDF 2015

Diabetic retinopathy

- Complications
  - Affects 40% of diabetics
  - Macrovascular
  - **Microvascular****
  - Others
    - Infections

Prevalence rate of any retinopathy in the adults with diabetes is~ 40%

Diabetes Canada



Problem

Therapy:

Current therapy for patients with diabetic macular edema and proliferative retinopathy:

Intravitreal injections of anti-VEGF.

Limitations:

frequent intraocular injections

Local or systemic adverse effects

unresponsiveness to anti-VEGF treatments .

Need: New Treatment

Diagnosis:

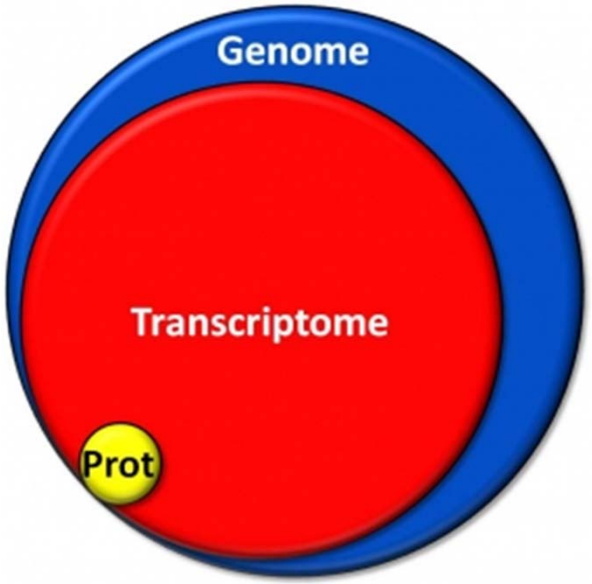
Limitations:

- Lack of blood based diagnostic markers of diabetic retinopathy
- Global challenge - difficulties in accessing specialists, trained and equipped for diagnosis and treatment .

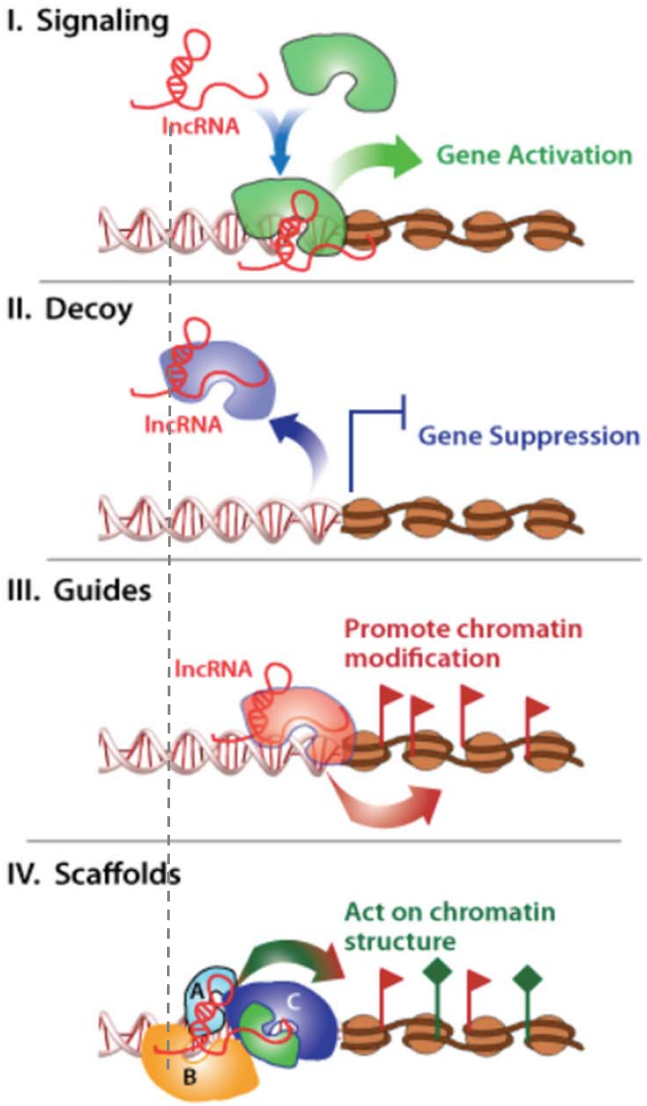
Need: Easily accessible quick and correct diagnostic test.

Specifically we explored long noncoding RNAs

lncRNA



- < 3% of the genome is protein-encoded – rest non-coding (ncRNAs).
- lncRNAs- resemble mRNA
- >200bp in length
- *cis*-acting or *trans*-acting
- One ncRNA may regulate multiple transcripts



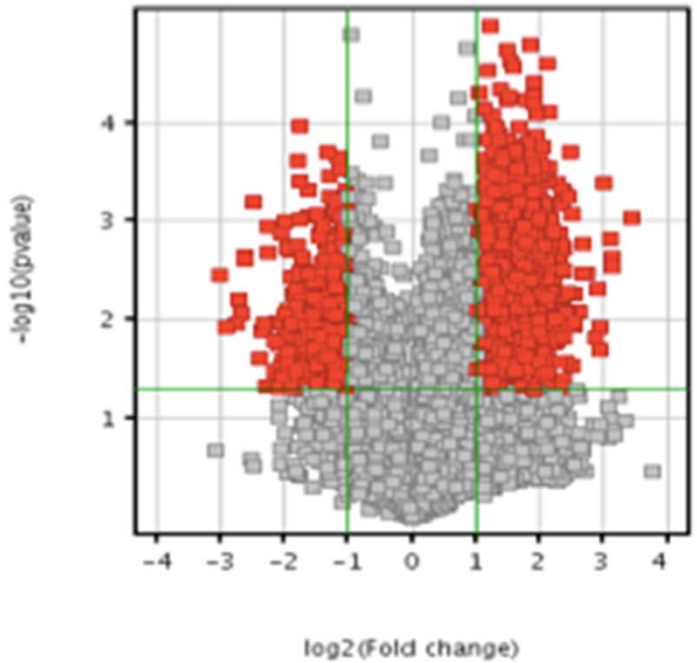
Wang KC and Chang HY. *Mol Cell*. 2011

DIAGNOSTIC PROJECT

lncRNA in diabetic retinopathy

- We have done extensive work on various lncRNAs and miRNA

(~25 peer reviewed publication on ncRNAs in diabetic complications, - extensive cell culture and animal experiments)



Glucose caused alterations of several lncRNAs in retinal endothelial cells

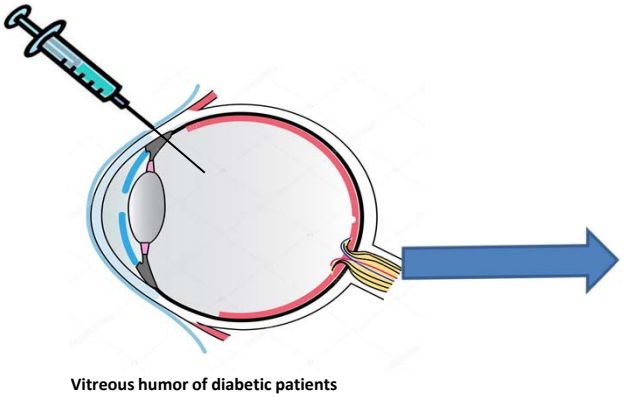
We examined the ones that may be important in vascular diseases

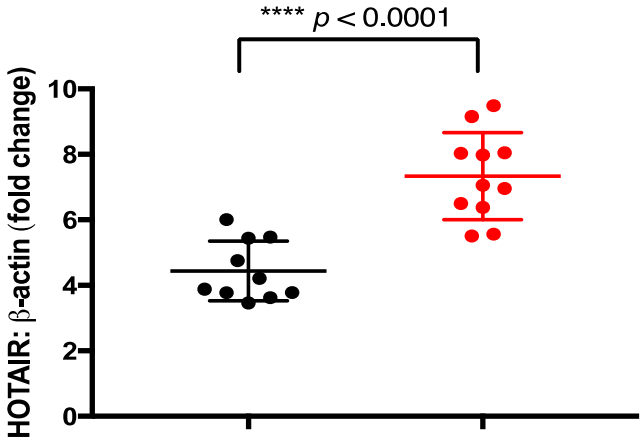
HOTAIR, ANRIL, H19, HULC, MALAT1, MEG3, MIAT, WISPER, ZFAS1

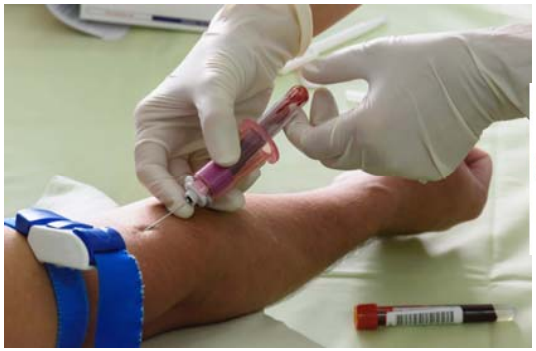
In this project:

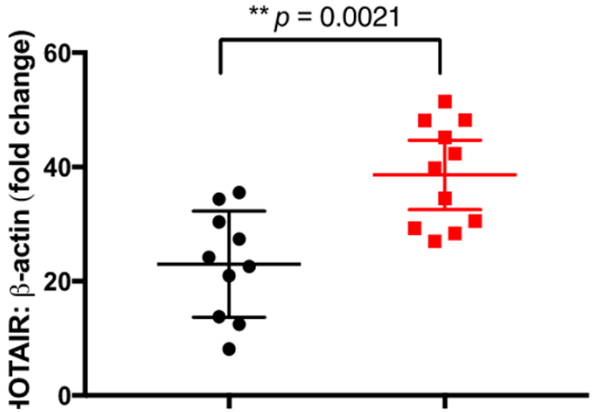
we focused on ***HOTAIR***

HOTAIR is upregulated in the vitreous and in serum in proliferative diabetic retinopathy patients





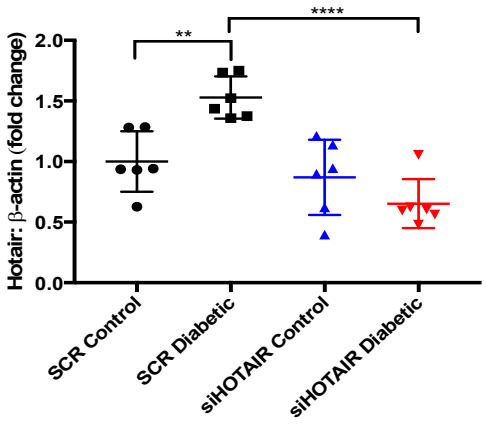




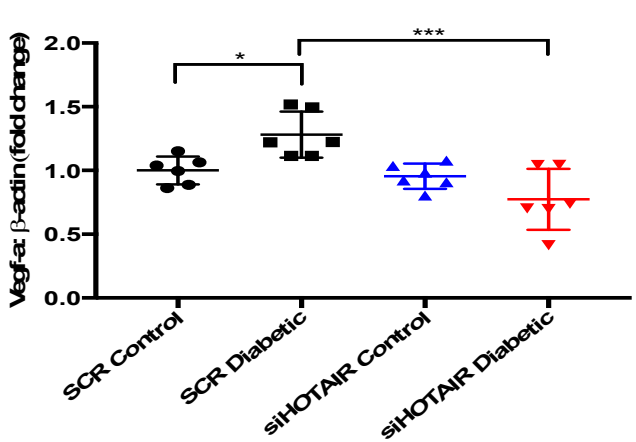
Serum and vitreous HOTAIR levels correlates in prolif. diabetic retinopathy patients

Intravitreal siHOTAIR prevents elevations of diabetes-associated molecules in the retina in animals

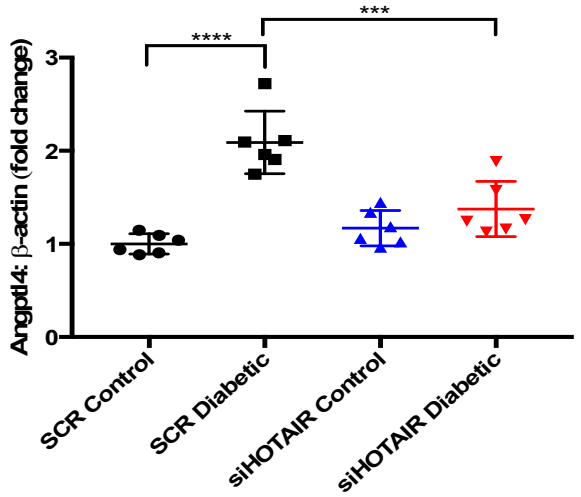
Hotair



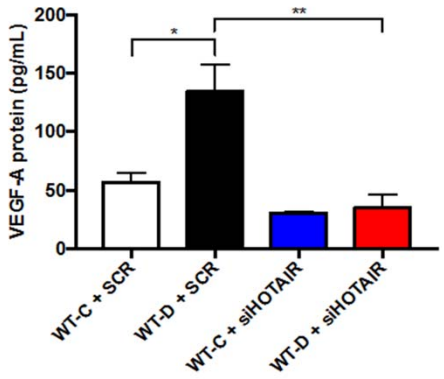
VEGF



Angptl4



VEGF Protein

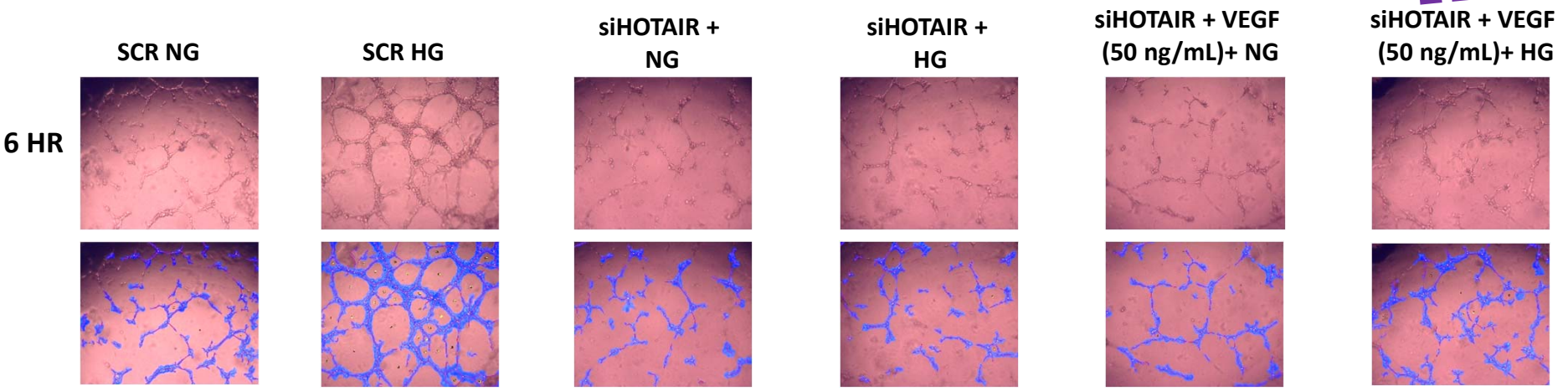


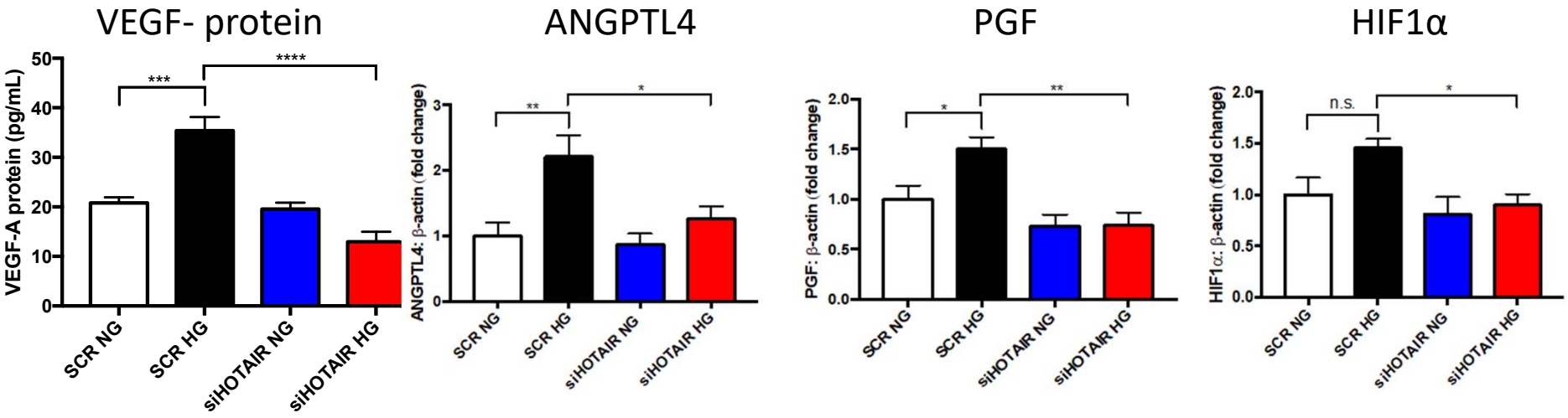
siHOTAIR also prevents elevations of a large number of molecules in the glucose exposed cells and in the retina of diabetic animals – **listed in slide 12**

- Weekly intravitreal siRNA injection for 1 month. No ocular or systemic toxicity were detected
- Alteration of other diabetes-associated and epigenetic molecules were also prevented

scr = scrambled and si= siRNA */**/*** = significant

Glucose increases HOTAIR which mediates angiogenesis through VEGF-A and others



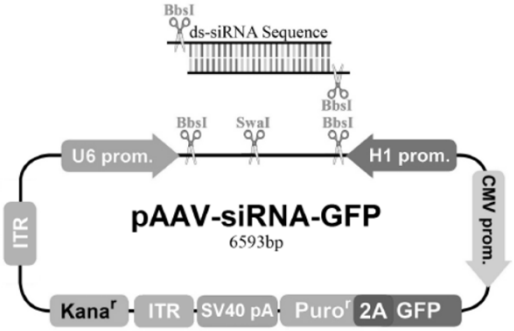


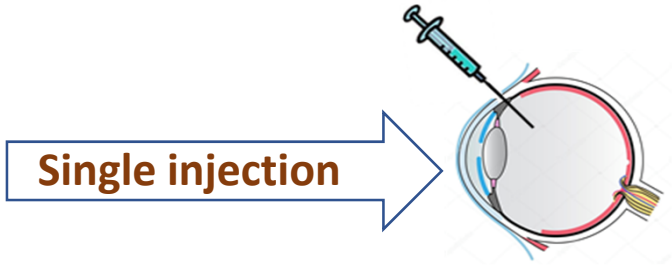
NG= 5mM glucose, HG=25mM glucose, si= siRNA, SCR=scrambled

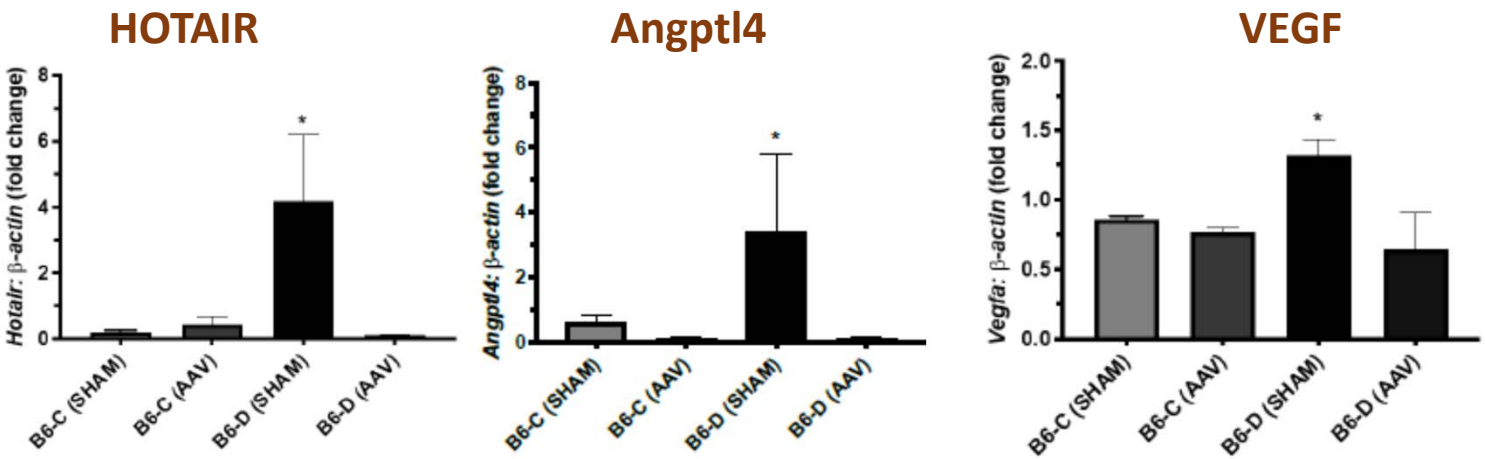
We carried out extensive mechanistic studies re: HOTAIR

Biswas et. al IOVS: 62:20, 2021

Long term effects can be achieved by a single injection of AAV2-siHOTAIR



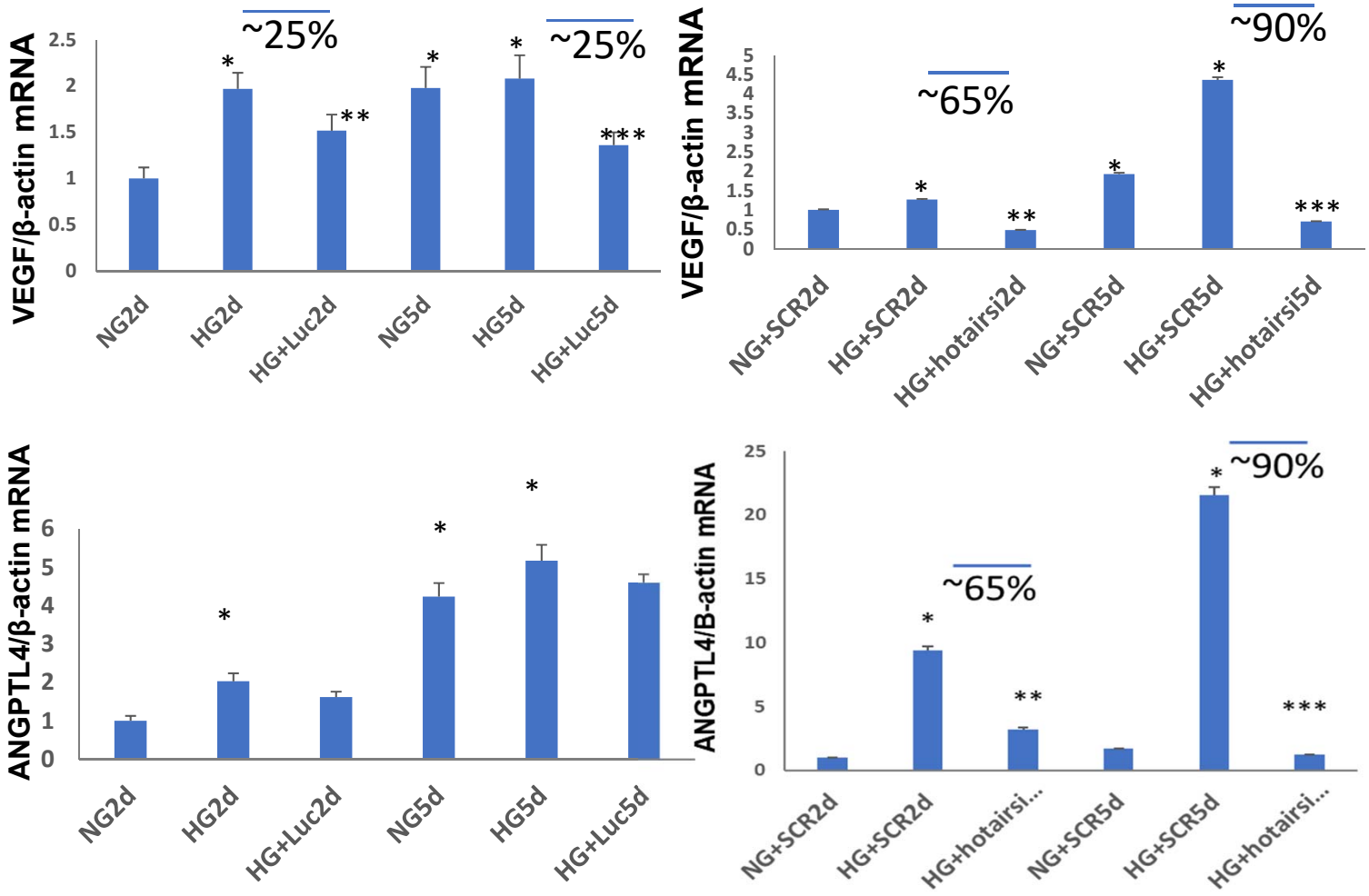




- A <u>single</u> intravitreal dose of AAV2-siHOTAIR prevented increase in retinal HOTAIR, Angptl4 and VEGFa levels in the diabetic mice at 1 month of follow-up.
- <u>No</u> ocular or systemic toxicity were seen.

C= non-diabetic controls, D= STZ induced diabetic, SHAM = Vehicle, AAV= AAV2 siHOTAIR, * = significant

siHOTAIR is a robust and long lasting blocker of angiogenic molecules compared to VEGF blocker



NG= 5mM glucose, HG=25mM glucose, 2d/5d= 2days/5 days, SCR=scrambled, luc= lucentis

HOTAIR Knockdown Prevents Glucose-Mediated Induction of :

DNA and oxidative damage:

PARP-1 and *cytochrome*

Epigenetic Mediators:

EZH2, *SUZ12*, *DNMT1*, *DNMT3A*, *DNMT3B*, *CTCF*, and *P300*

Inflammatory mediators:

Il1B, MCP-1

Multiple Angiogenic Factors:

VEGF-A, *ET-1*, *ANGPTL4*, *PGF*, *HIF-1α*

These are all important mediators of diabetic retinopathy.

At the functional level targeting *HOTAIR* prevents:

Retinal Mitochondrial dysfunction

Retinal Dysfunction

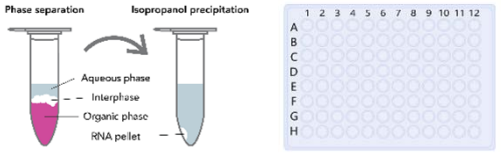
permeability, angiogenesis

Biswas et. al IOVS: 62:20, 2021

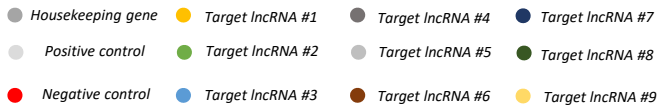
DIAGNOSTIC PROJECT

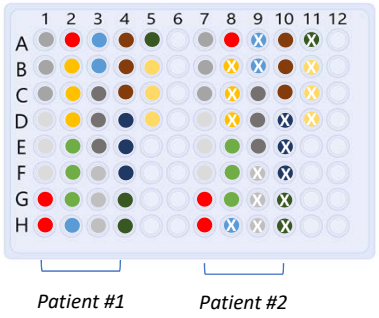
1. Family physicians sees the patient

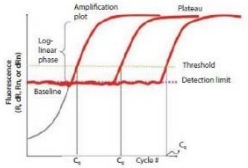
2. Blood work is performed, Serum is collected



3. Total RNA is extracted and RNA is prepared for qPCR lncRNA panel.



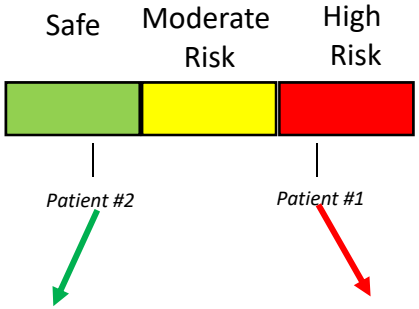




4. qPCR is performed using the lncRNA panel.

5. qPCR results are analyzed for the expression values for each target lncRNA.

DR Severity/Probability Scale

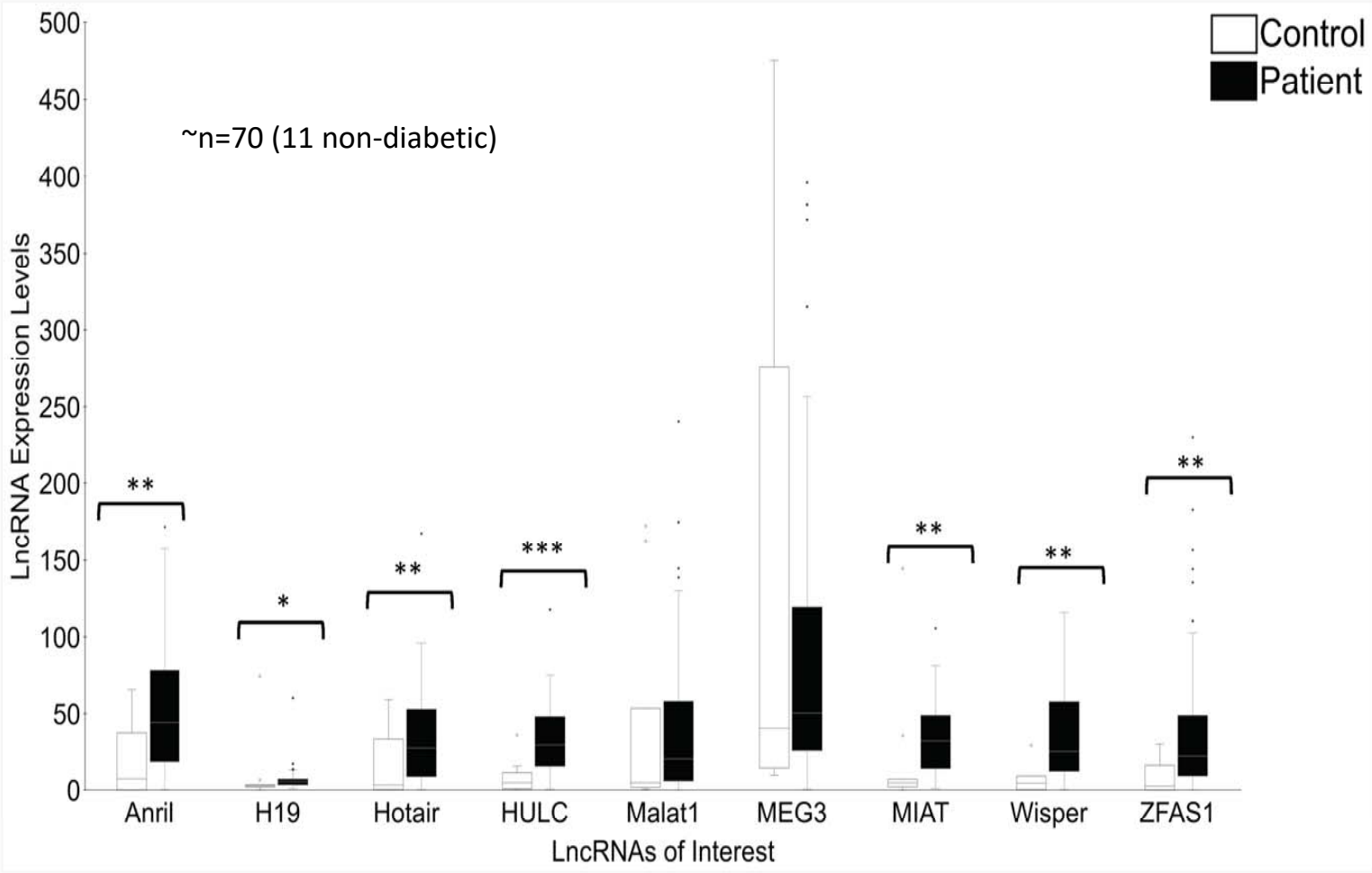


Follow up by family physician

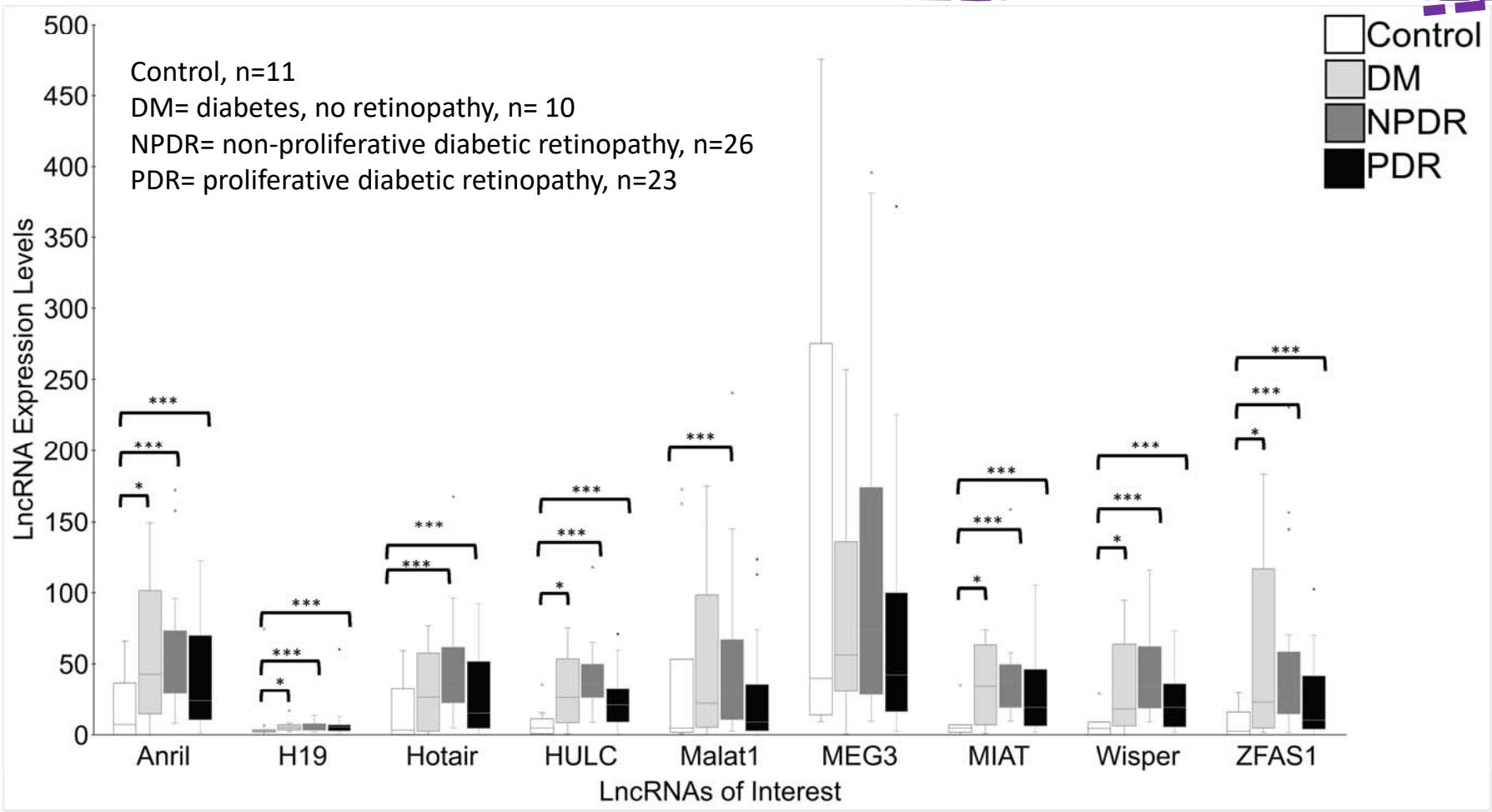
Referred to ophthalmologist

initial study – n=21 prolif. diabetic retinopathy patients (including 10 controls) – good correlation of vitreous and serum levels of HOTAIR and 8 other lncRNAs

lncRNA as a biomarker for diabetic retinopathy



lncRNAs as biomarker for diabetic retinopathy



- **Using various combinations of serum lncRNA levels: we can identify a significant number of retinopathy patients** (*FRONTIERS IN ENDOCRINOL.* – https://doi.org/10.3389/fendo.2022.851967)
- **Larger study has been initiated**

Summary of findings

Therapeutics:

- HOTAIR in increased in animal and human diabetic retinopathy
- HOTAIR is responsible for several lesions of diabetic retinopathy
- Silencing HOTAIR prevents pathologic changes in the animals
- Potential therapy for DR?

Diagnostic

- HOTAIR in serum and is increased and human diabetic retinopathy
- A panel of lncRNAs can be used as biomarker.

Current diagnostic and therapeutic modalities

Diagnostic tools:

- Optical coherence tomography (OCT)
- Fluorescein angiogram
- Wide-field retinal imaging
- Electroretinography (ERG)

Treatments:

- Intravitreal anti-VEGF and steroid agents for PDR
- Vitreoretinal surgery
- Laser photocoagulation
- Maintaining near-normal sugar levels

Limitations:

- Lack of blood based diagnostic markers
- Global challenge - difficulties in access .

Limitations (VEGF):

frequent intraocular injections
Adverse effects
unresponsiveness to anti-VEGF

Our approach potentially overcomes these limitation

Story about patent

Diagnosis and Treatment of Chronic Diabetic Complications Using long non-coding RNAs as Targets

US Patent Application number: 63/048389, EFS ID39918968, 07/06/2020
PCT international patent application No. PCT/CA2021050924 (July, 6 2021)

Main claims:

- HOTAIR may be used for RNA based therapy (Diabetic retinopathy and other conditions)
- A panel of lncRNA may be a diagnostic tool for diabetic retinopathy

Advantages and Benefits

Market Size *(IDF 2021)*

Diabetic Population 537 million
+541 million IGT

uses 9% of global healthcare $ US$966 billion

Target Market

Current diagnostic tools

Ophthalmoscopy
FFA
OCT

Difficulties

- ✓ Difficult to determine early cellular changes
- ✓ Highly trained individuals
- ✓ Resources

Competition – none

-No RNA based treatments are available

No blood RNA biomarkers are available

Benefits to Stakeholders

Physicians/Patients

- ✓RX- Longer, robust effects with fewer adverse events
- ✓DX - Early diagnosis and progression monitoring

Policy makers

- ✓ Screening tests save health care $

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