Exploring the metabolic changes occurring in *in vitro* and *in vivo* models of diabetic retinopathy

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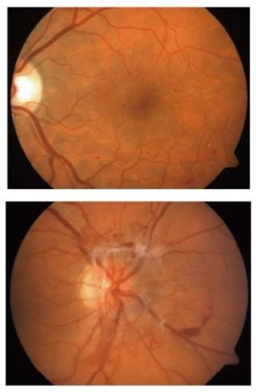
INTRODUCTION

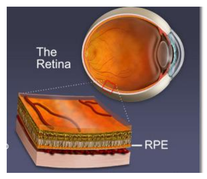
Diabetic retinopathy (DR) remains as a leading cause of blindness in developed countries.
This complex pathology involves neurodegeneration and vasculature of the inner retina.

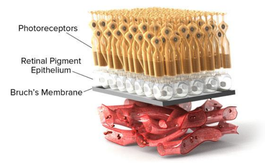
DR stages:

Early diabetic retinopathy (non-PDR)
Developing of micro-vascular anomalies.

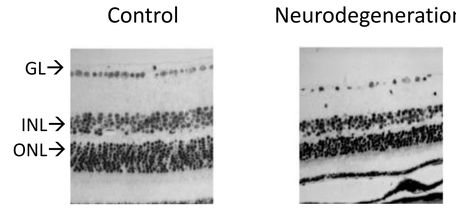
Proliferative diabetic retinopathy (PDR)
Neovascularization process leading to occlusion of retinal vessels (hypoxia) and the loss of integrity of the inner blood-retinal barrier.







Morphological changes of retina



Ganglion cell layer (GL), inner nuclear layer (INL) and outer nuclear layer (ONL)

Current **treatments** for DR are applicable only at advanced stages of the disease, when the blood vessels proliferate PDR and they associate with significant adverse effects.

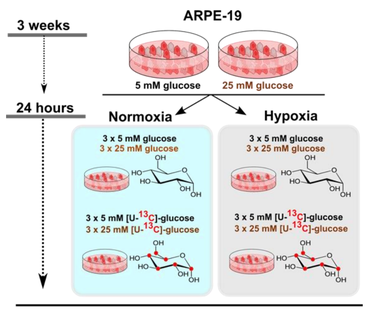
GOAL

Identify deregulated metabolic pathways leading to DR based on the study of *in vitro* hyperglycemic pseudohypoxic conditions and *in vivo* early stages of diabetic retinopathy.

METHODS

1. Samples

In vitro model: human retinal pigment epithelium cell line (ARPE-19).



5 mM glucose and normoxia (N5; control)
5 mM glucose and hypoxia (H5)
25 mM glucose and normoxia (N25)
25 mM glucose and hypoxia (H25)

In vivo animal model

	High fat diet	Irs2-/- mutant
Systemic phenotype	Insulin resistance No hyperglycemia No diabetes	Insulin resistance Hyperglycemia Diabetes
Retinal phenotype	Little neurodegeneration No vascular lesions	50% photoreceptor degeneration No vascular lesions
Samples	Retinas (7 HFD + 7 controls)	Retinas (10 Irs2(-/-) + 14 controls)

2. Untargeted metabolomics

- Mass profiling (^{12}C)
- Isotope tracking (^{13}C) using geoRge an in-house software (ref 2)

600 MHz Bruker Avance III
+cryoprobe
+SampleJet

Agilent
6550 LC-qTOF MS

Agilent
7200 GC-qTOF MS

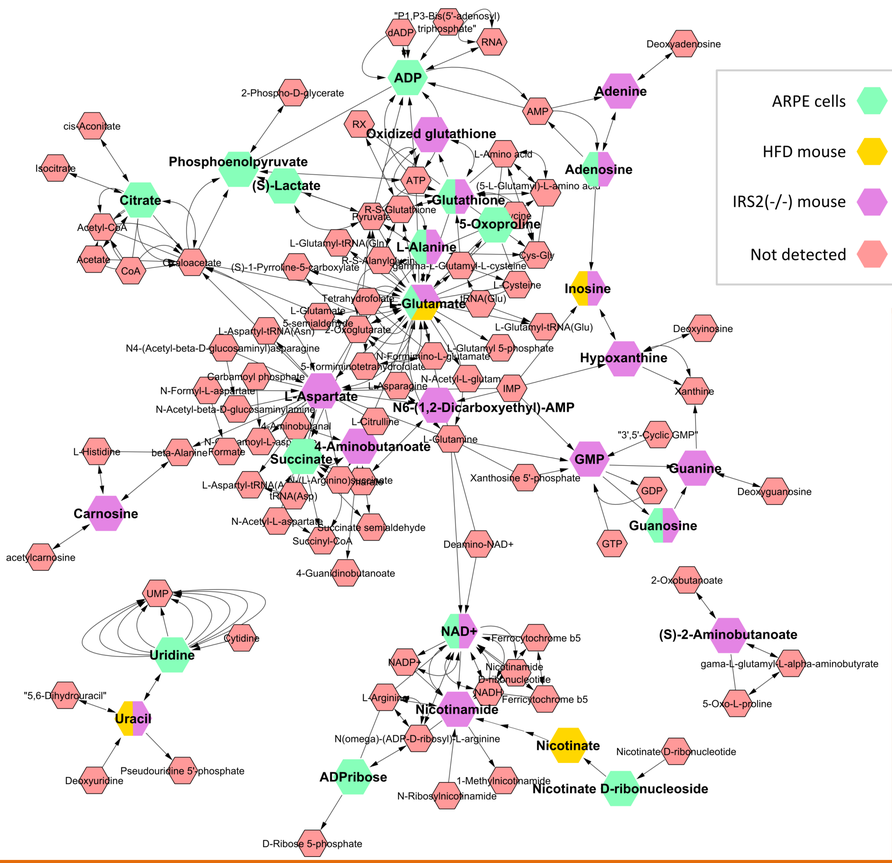
3. Data integration and network visualization

MetScape (plugin from CytoScape)
Input data: metabolites statistically significant (pval<0.05 and abs(FC)>1.5)

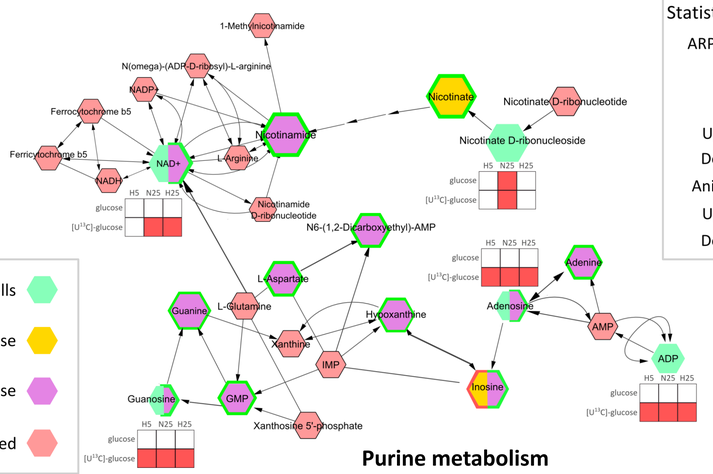
Cytoscape

RESULTS

Network integration of the cell and animal models:
The metabolic network represents all the connections between the altered metabolites detected in the ARPE cell model and in the animal models (HFD and Irs2(-/-).
Irs2(-/-) is the model that represents the highest impact in the network.



Nicotinate and nicotinamide metabolism



Purine metabolism

Statistics:
ARPE cells
H5 N25 H25
glucose
[U13C]-glucose
Up regulated versus control
Down regulated versus control
Animal models
Up regulated versus control
Down regulated versus control

Subnetworks: nicotinate and nicotinamide and purine metabolisms.

- The Irs2(-/-) models is characterised by a downregulation of all the metabolites detected.
- HDF model only presents two metabolic alterations: upregulated inosine and downregulated nicotinate.
- In ARPE cells metabolic flux analysis reveals changes in ^{13}C incorporation levels in several metabolites while ^{12}C pool levels keep constant. In general, the major flux alterations are due to high glucose concentrations (N25 and H25) rather than low O_2 levels (H5).

DISCUSSION

Adenosine and inosine are considered as a potential immunomodulatory and neuroprotective agent. Adenosine is rapidly metabolized to inosine, and inosine mimics the protective effect of adenosine. This fact is in line with the results obtained from the neurodegenerative Irs2(-/-) model. Low levels of adenosine and inosine would not protect the retina from neurodegeneration. Contrary, the HFD mouse presents high levels of inosine. The upregulation of inosine could be explained as a response to insulin resistance with the aim of protecting the neouroglial cells. The detection of this changes in human patients could contribute to apply new therapeutic targets to avoid advanced stages of diabetic retinopathy (ref 1).

REFERENCES

1. Navarro et al. *submitted*.
2. Capellades et *submitted.*