Using Artificial Intelligence and Biological Data To Influence Stem Cell Differentiation

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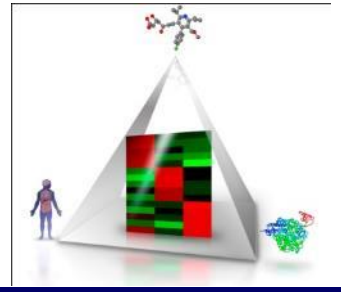


Any statements made during this talk are in my capacity as an academic

Short preamble about Artificial Intelligence

- Statistics, machine learning, bioinformatics, cheminformatics, … those fields existed for a long time
- There is no magic to it, they all just encompass the analysis of quantitative data in different ways
- Recently image and speech recognition have seen tremendous progress due to ‘deep learning’ (particular types of neural networks), which now diffuses into other areas
- But chemical biology/drug discovery are very different, doesn’t entirely translate to this field

- *You should analyze your data, but what you can gain from it depends largely on the data, much less so on the method*

Review articles
(accessible to general scientists, open access)

"Artificial Intelligence in Drug Discovery – What is Realistic, What are Illusions?"

Part 1: Ways to make an impact, and why we are not there yet

Part 2: A discussion of chemical and biological data

Andreas Bender and Isidro Cortes

Drug Discovery Today 2021

http://www.DrugDiscovery.NET/AIReview

"How to Lie With Computational Predictive Models in Drug Discovery"

http://www.DrugDiscovery.NET/HowToLie

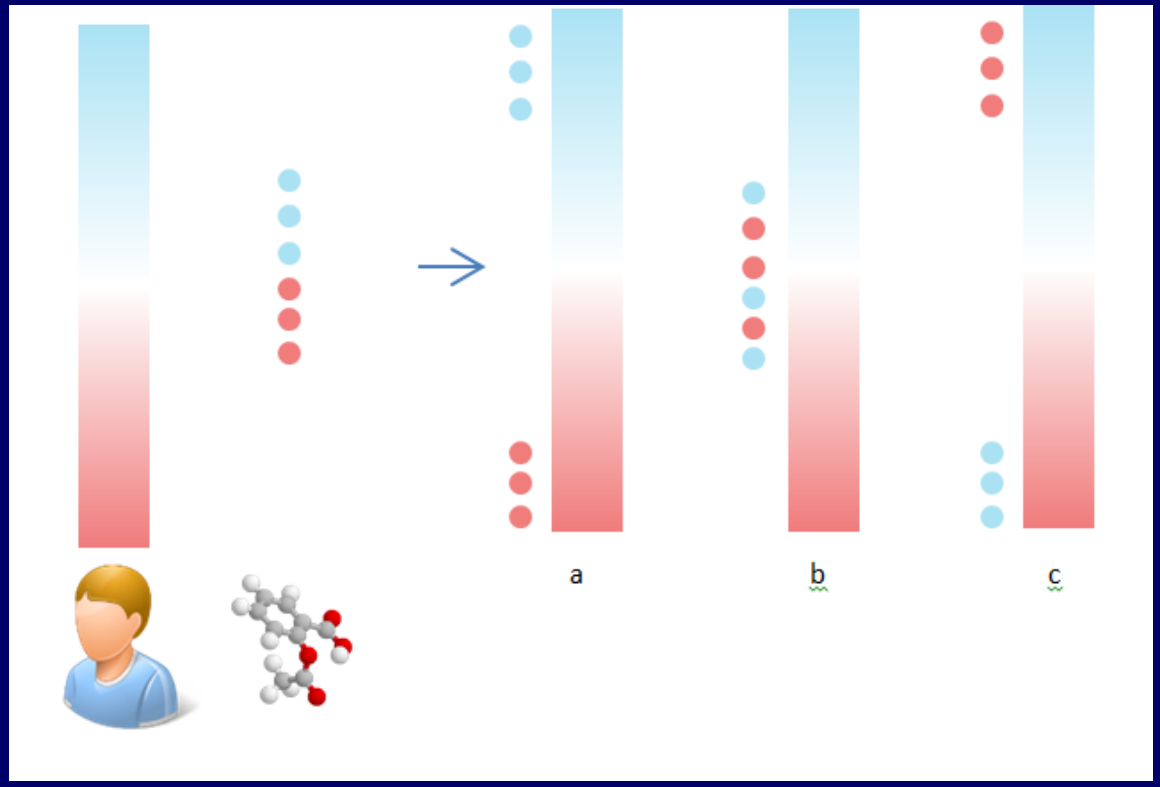
Outline

- How gene expression data and computational methods can be used for compound selection

- Applications
  1. Differentiation therapy in cancer
  2. Differentiating stem cells to cardiomyocytes
  3. Improving success rates in cloning / somatic cell nuclear transfer (SCNT)

“BioStateConverter” (work of Yasaman KalantarMotamedi, experimental work performed by Dr Nasr + Team from Royan)

- Compound-Indication mapping *via* gene expression data
- **Key Idea: compound treatment shifts system from state A to state B (diseased to healthy; stem cell to differentiated cell, etc.)**
- Needs gene expression data of state A and state B; matches this with compound-induced gene expression data
- Details depend on implementation



Gene expression changes of person, diseased vs healthy, and compound (blue up, red down)

Compound c matches best (in this case), has opposite profile to disease

Data Sources available

- *Diseases, cell states*: Gene Expression Omnibus (GEO), and others

- *Compounds*
  - ConnectivityMap (1,300 compounds to Affymetrix chips)
  - LINCS (12,000 compounds to 1,000-gene expression signatures)

- We can match any compound we have gene expression data for to any indication we have gene expression data for

- Caveats: Cell lines/systems are different, time points, concentrations

Case studies applied to cellular differentiation

1. Differentiation therapy in leukemia

2. Differentiating stem cells into cardiomyocytes

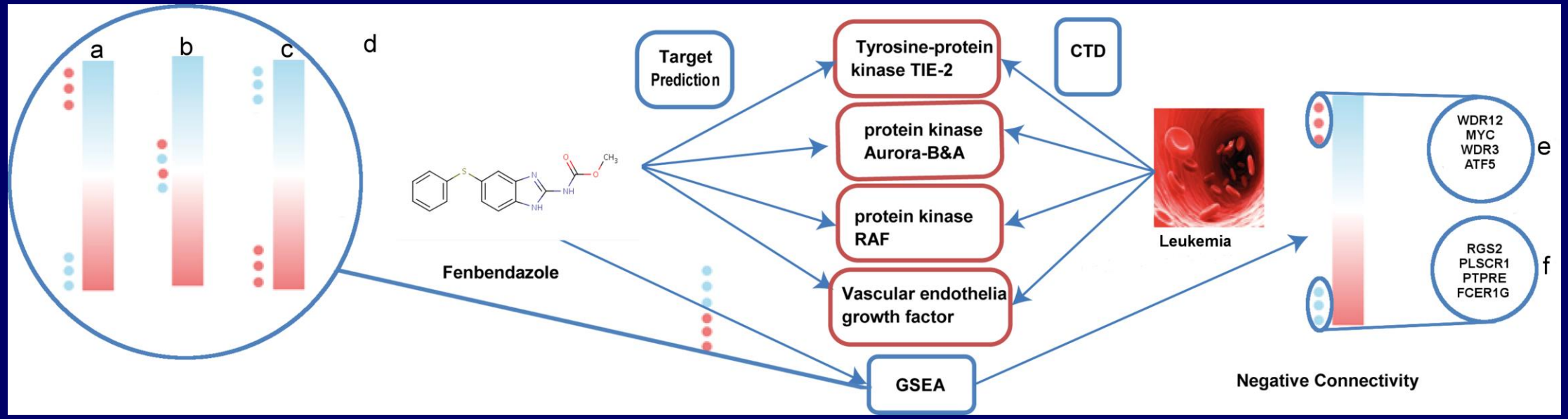
3. Selecting small molecules to increase reproduction rates in cloning

1. Differentiation therapy in leukemia

- Transcriptional drug repositioning and cheminformatics approach for differentiation therapy of leukaemia cells.
- Y KalantarMotamedi, F Ejeian, F Sabouhi, L Bahmani, AS Nejati, AM Bhagwat, AM Ahadi, AP Tafreshi, MH Nasr-Esfahani, A Bender – Sci Rep (2021) 11, 12537

Aim and setup

- Differentiation therapy has advantages compared to chemotherapy (eg irreversible effect, rapid clearance of tumour bulk)
- In acute myeloid leukaemia (AML) cells, differentiation is blocked in cellular maturation stage
- Assumption: We can select small molecules to overcome differentiation block
- Used combination of gene expression data and on-target predictions



- States used: disease signature (HL60 leukaemia cells vs. granulocyte)

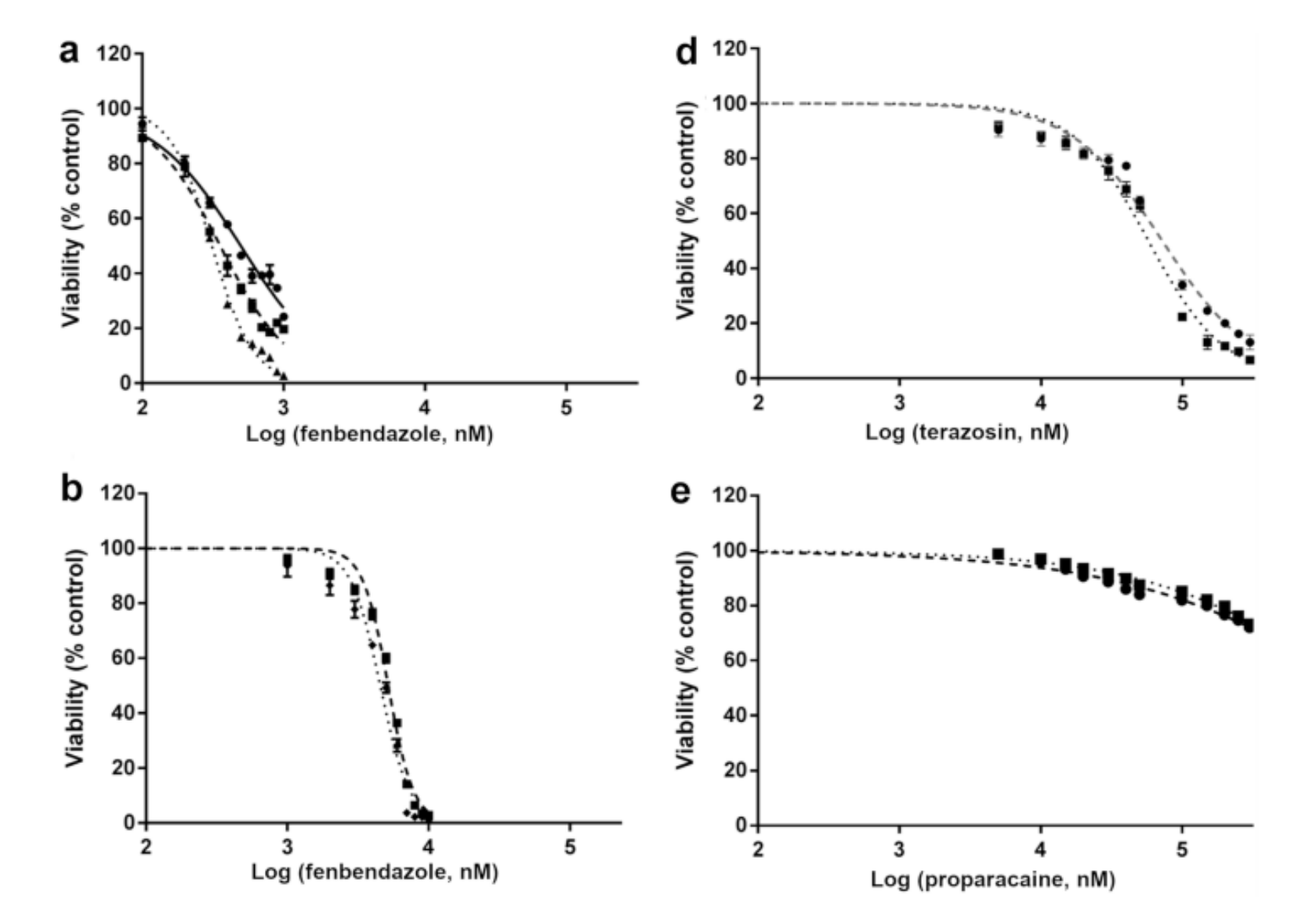
Retrospective validation, compound selection

- Applied connectivity mapping/BioStateConverter procedure as described in the introduction

- 20 out of the 30 highest-ranked compounds from CMap with negative connectivity were supported by literature according to their relevance to leukaemia

- Proxymetacaine (ranked 18), fenbendazole (ranked 20) and terazosin (ranked 4) were selected to be tested *in vitro* on HL60 leukaemia cell line

Fenbendazole shows sub-uM LC50 on HL60 cells



LC50 0.5uM on HL60 cells

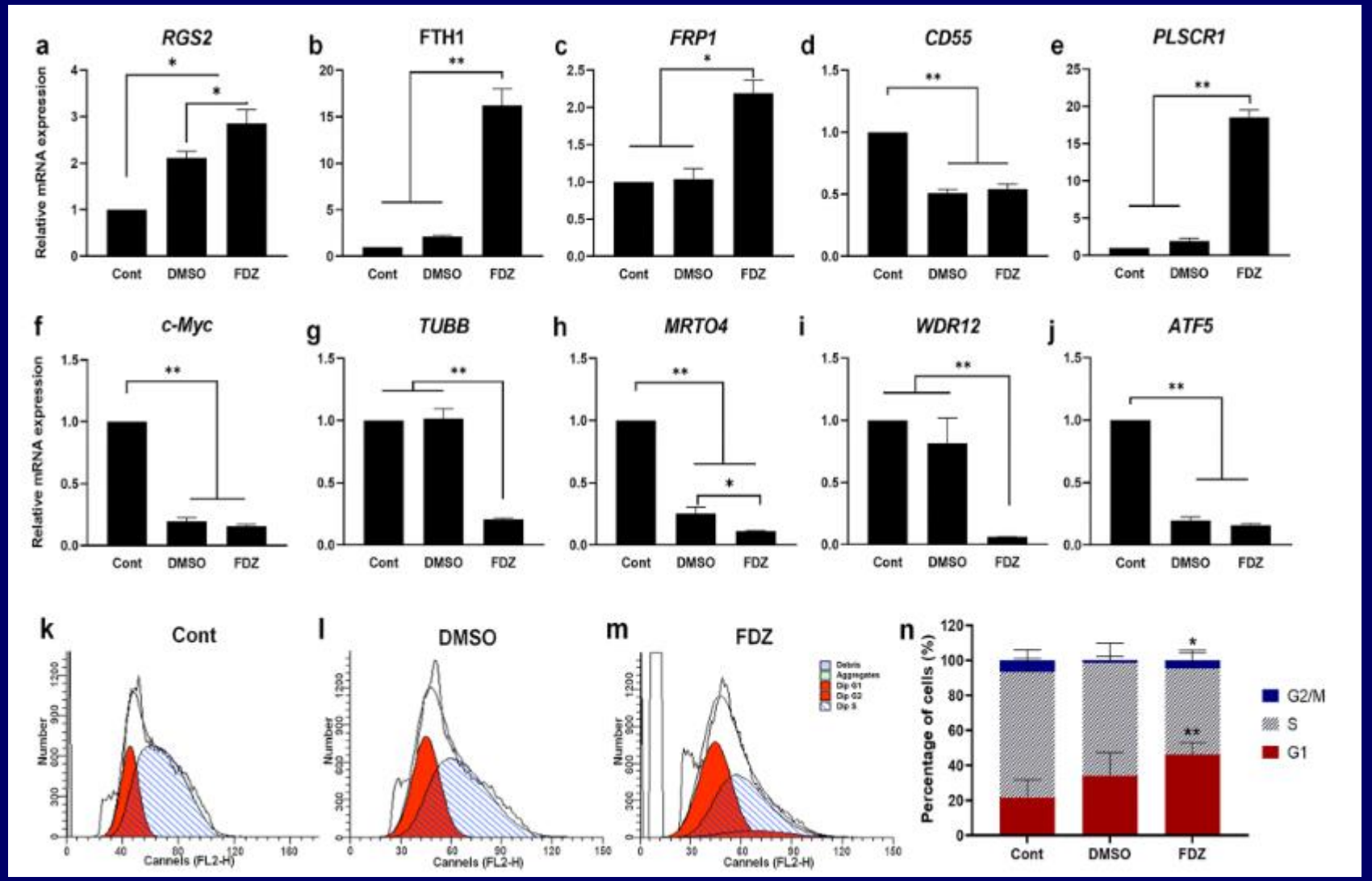
5uM LC50 on BMSCs

~10-fold selectivity

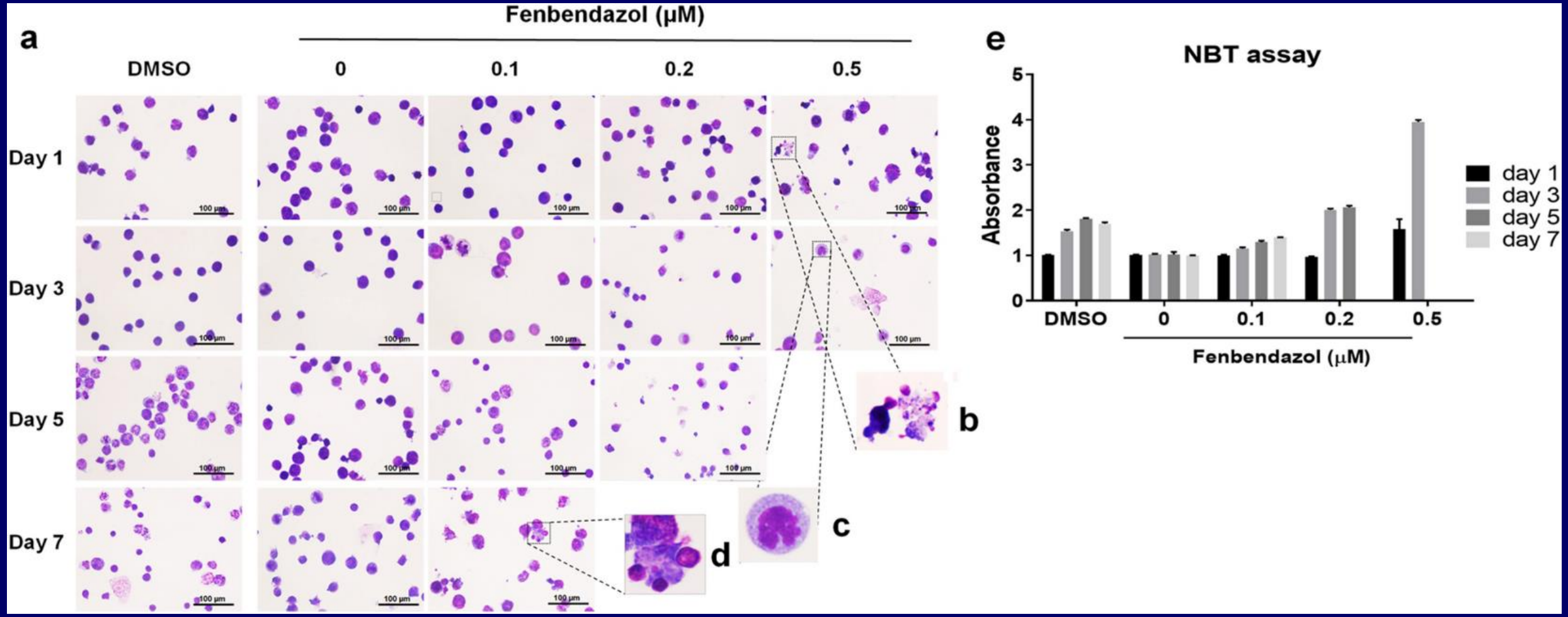
Mechanistic analysis matched expected mode of action to a large extent

Expected upregulation of genes a-e, downregulation of genes f-j

More detailed analysis in paper

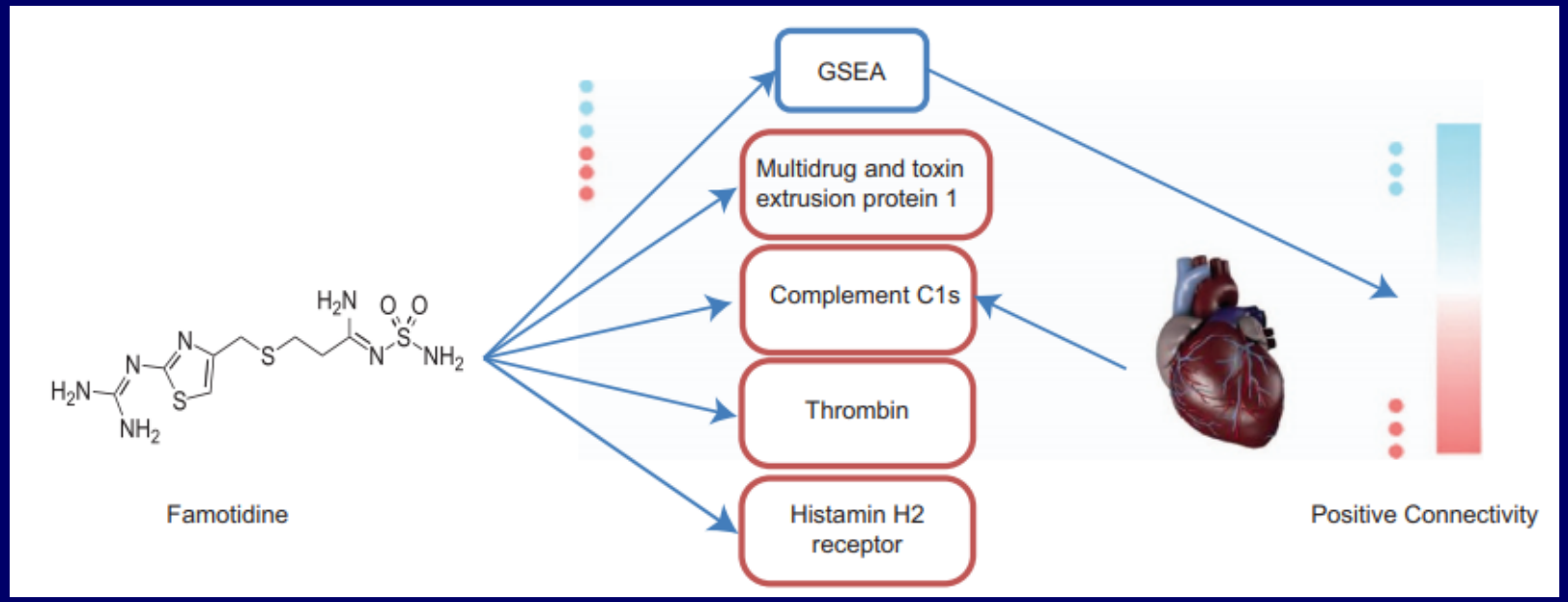


At low concentrations also visual inspection and Nitro Blue Tetrazolium (NBT) reduction assay consistent with differentiation to granulocytes



2. Systematic selection of small molecules to promote differentiation of embryonic stem cells and experimental validation for generating cardiomyocytes

- Undifferentiated human embryonic stem cells vs adult ventricular cardiac tissue
- Y KalantarMotamedi, M Peymani, H Baharvand, MH Nasr-Esfahani, A Bender – Cell Death Discovery (2016) 2, 16007
- Combined gene expression/target-based approach



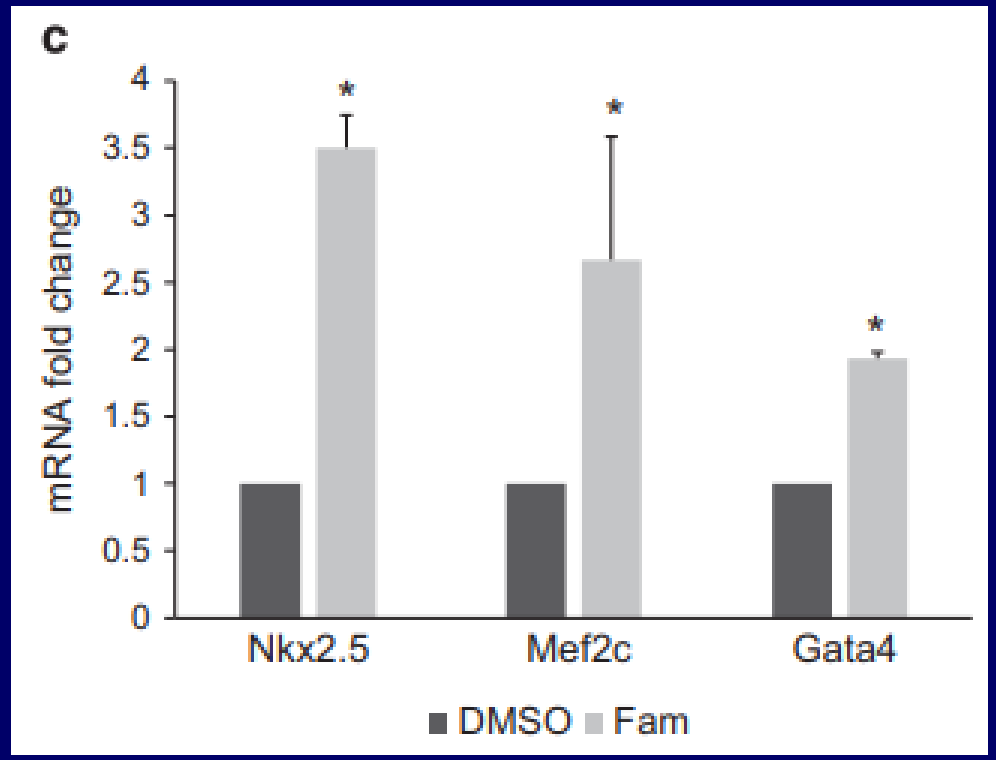
Retrospective validation and compound selection

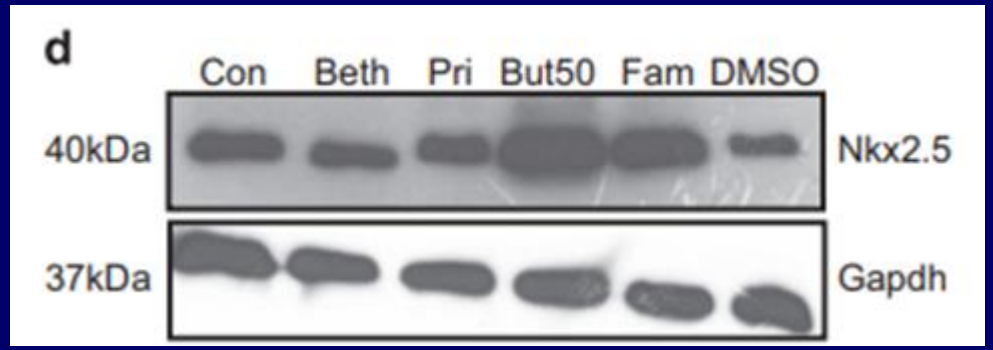
- Meglumine (rank 9 out of 6100), Troglitazone (rank 17) and *α*-Estradiol (rank 20) show literature evidence of related activity
- Meglumine capable of differentiating bone marrow mesenchymal stem cells to cardiomyocytes
- Troglitazone induces heart cell proliferation
- Estradiol demonstrates cardioprotective effect against cardiac injury
- Etc.

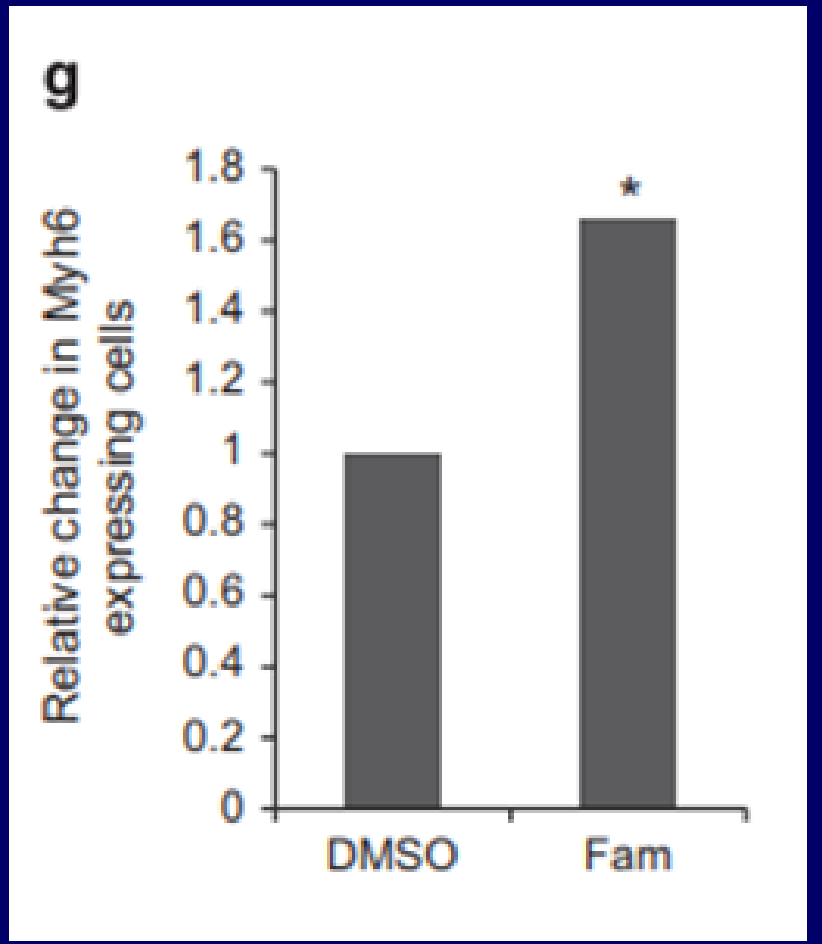
- Bethanechol (rank 2), Prilocaine (rank 4), Famotidine (rank 12) and Sodium Phenylbutyrate (rank 18, Butyrate) selected for experimental validation

Enhancement of cardiomyocytes during Cardiac Progenitor Cell (CPC) formation (day 7)

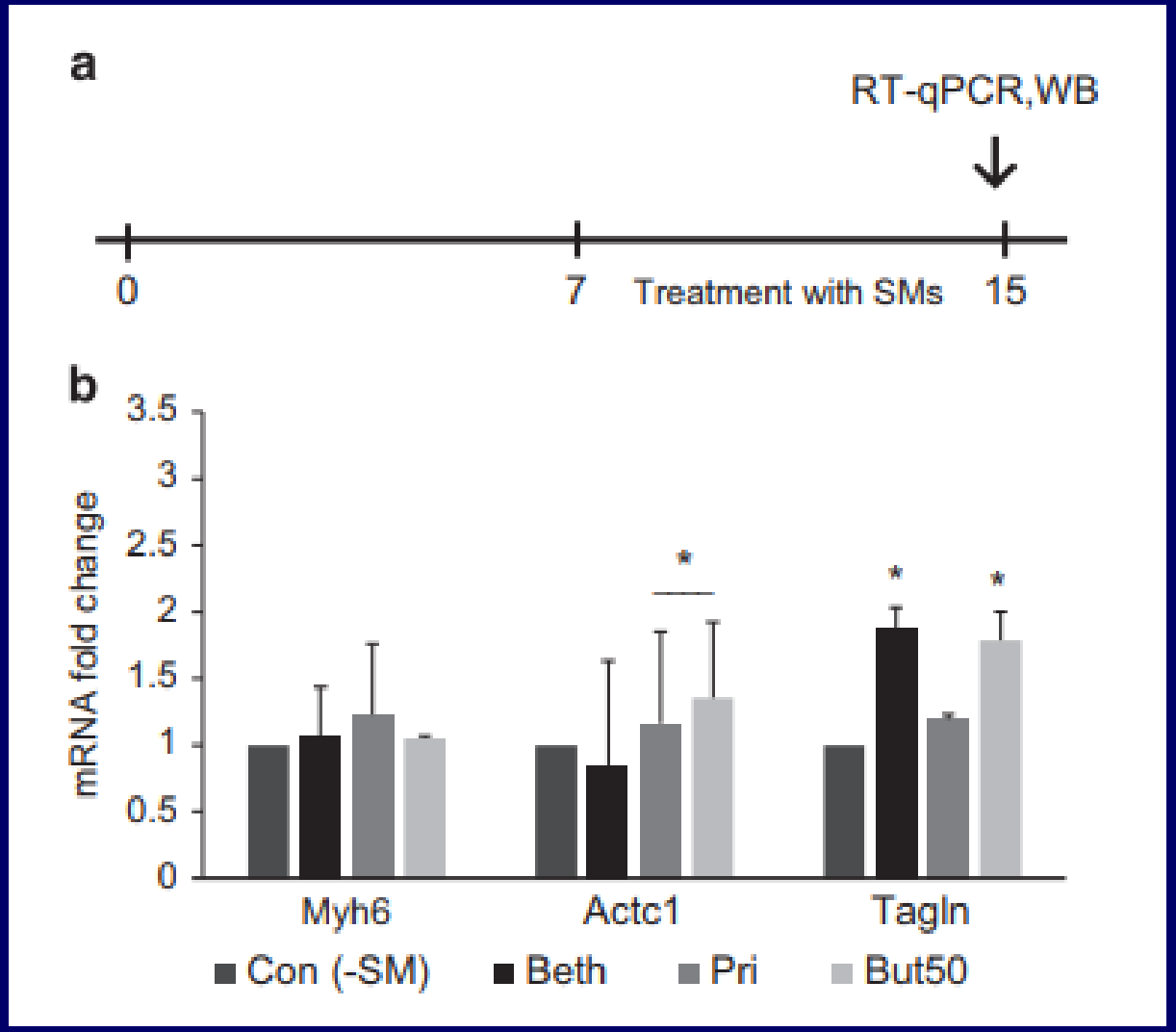
Cardiac markers upregulated (RT-PCR), especially for Famotidine (c), also on protein level (WB, d); more cells expressing Myh6 (by flow cytometry, g)

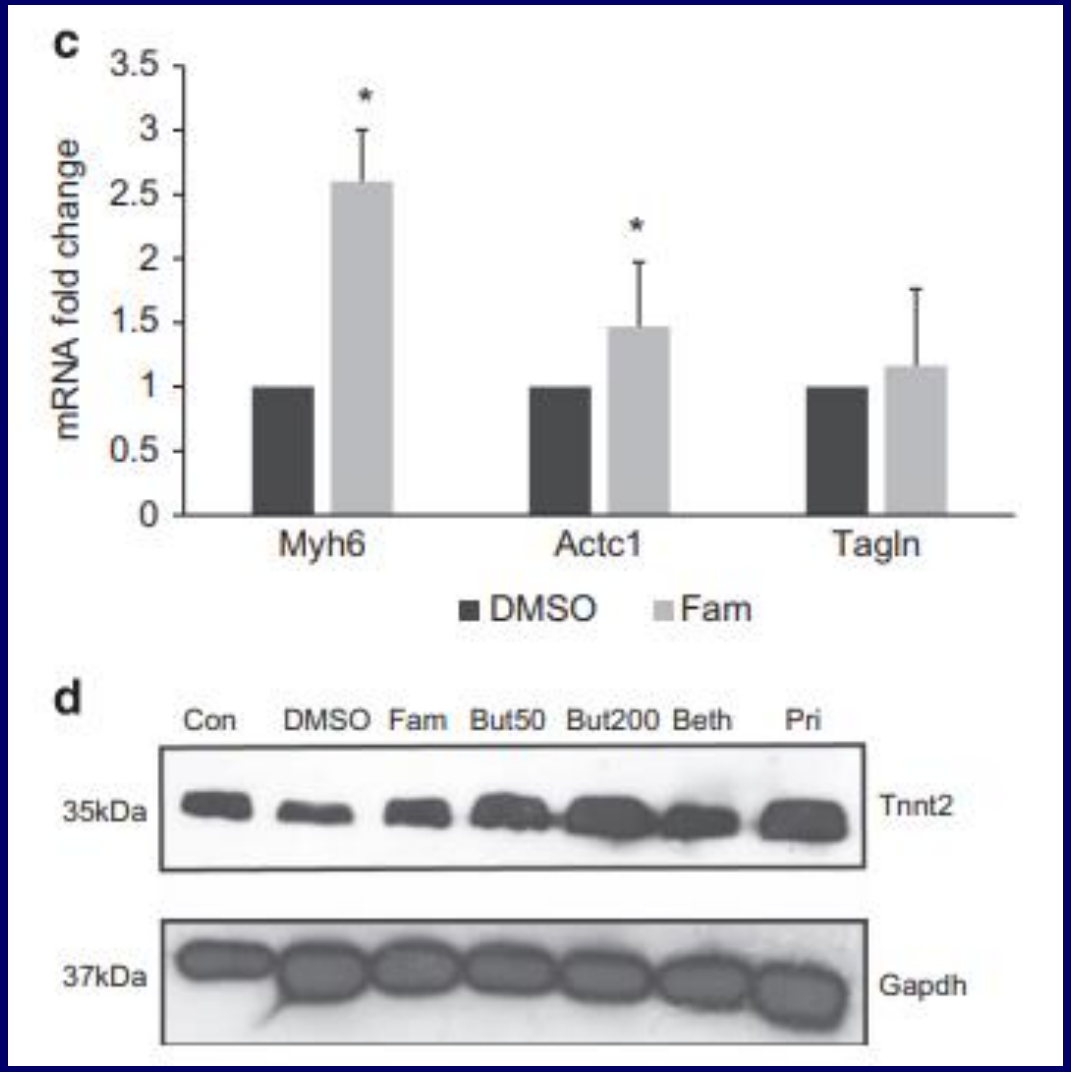






Effects also observed post-CPC formation (day 15)





Famotidine antihistamine, clinical reports have shown impact on cardiac regeneration

- Famotidine reduced infarct size of cardiomyocytes in mice after ischemia/reperfusion injury or permanent ischemia
- Effects had been associated to the anti-histamine effects of Famotidine rather than its role in promotion of cardiac differentiation

Kim J, Ogai A, Nakatani S, Hashimura K, Kanzaki H, Komamura K et al. Impact of blockade of histamine H2 receptors on chronic heart failure revealed by retrospective and prospective randomized studies. J Am Coll Cardiol 2006; 48: 1378–1384.

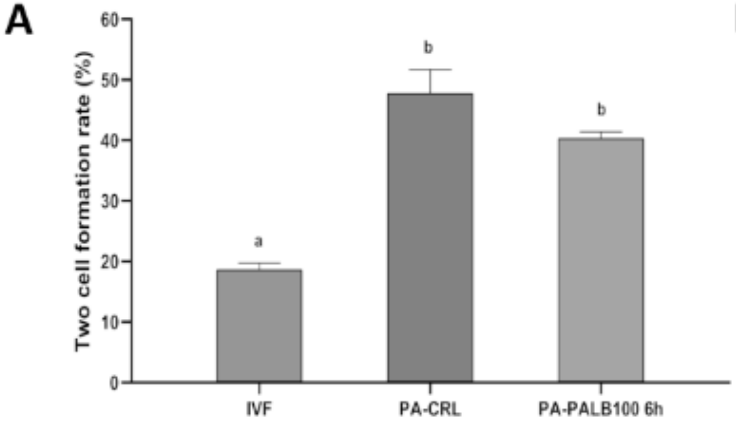
3. Application to cloning/SCNT Reproduction

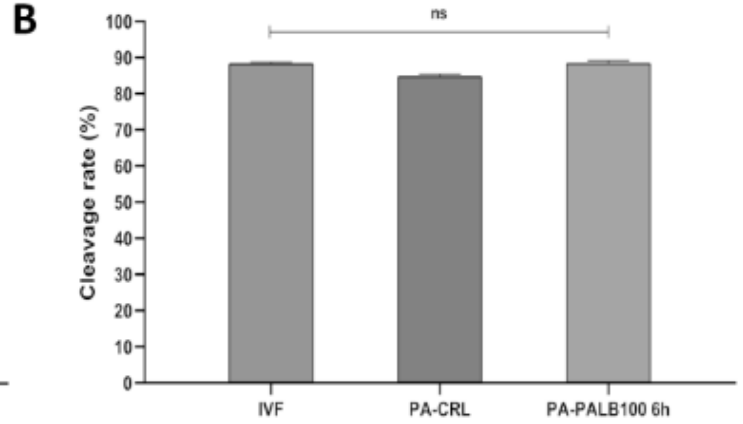
- Somatic Cell Nuclear Transfer (SCNT) efficiency is low
- Problem is abnormal transcriptional reprogramming (different biological nature of transferred somatic cells compared to gametes, the sperm and the oocyte during fertilization)
- HDACs, DNA methyltransferase inhibitors used to improve situation

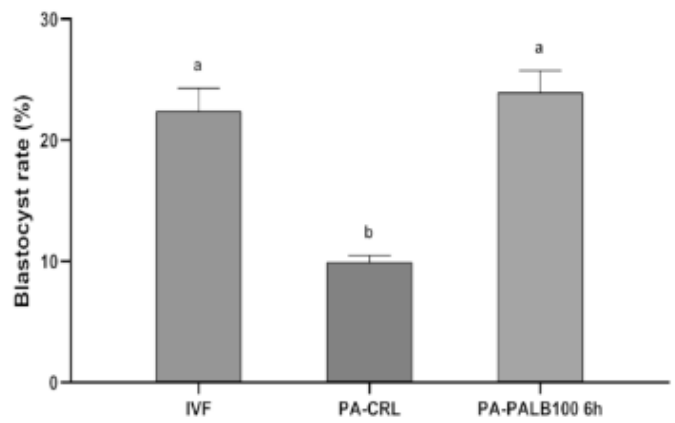
- Here aim is to find new small molecules to improve success rates
- IVF vs SCNT embryos as selection criterion (IVF has higher success rates)
- Selection of CDK4/6 inhibitors Palbociclib and Fenretinide, followed by optimization of protocol

IVF vs Parthogenic vs Treated: Two-cell formation rate, cleavage rate, blastocyst rate

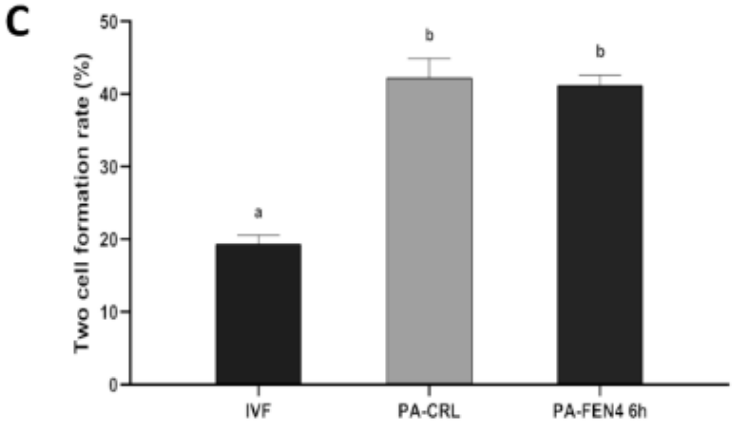
Palbociclib

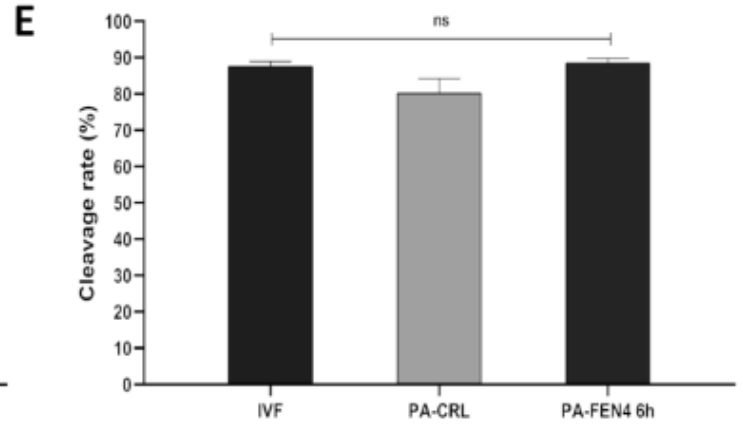


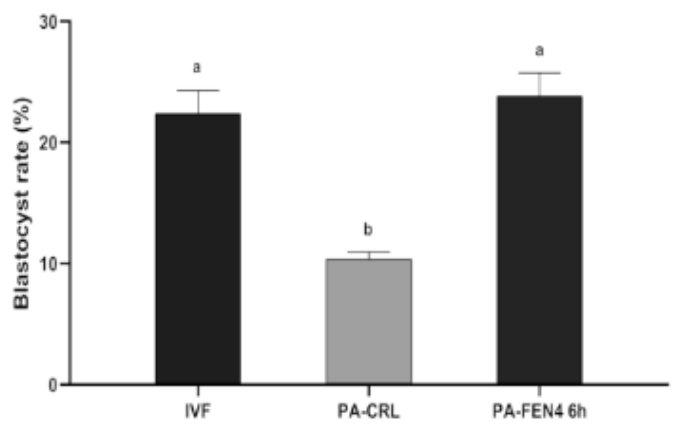




Fenretinide







Conclusion

- Palbociclib (100 nM for 12 h) and Fenretinide (16 µM for 6 h) prolonged time of first cleavage in treated SCNT embryos
- Also increased cleavage rate after 72h and improved developmental competence of SCNT derived embryos similar to those of IVF embryos *in* vitro

Overall Conclusions

- We can successfully use gene expression data, and algorithms, to select small molecules to influence stem cell differentiation

- Feel free to get in touch if you need, very happy to work with experimentalists and to put algorithms to good use

- Thank you to Dr Yasaman KalantarMotamedi, Dr Nasr + Team for the very supportive and successful collaboration

Thank you for listening!
Any questions?

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