Retinoic Acid Treatment Affects Gene Expression of Immature Neuronal Markers and Up-Regulates SNAP25 Expression in SH-SY5Y cells

Eline Poinsignon-Clavel
1006765528
HMB310 UofT

INTRODUCTION

- SH-SY5Y cells (neuroblast, immature neuron) [1]
  - Epithelial structure, grow in clumps, not fully differentiated when used for qPCR
  - Neuronal model

What is RA?

- Active form of vit A
- **Increases cell differentiation**
- Decrease cell proliferation
  - Can suppress tumors
- Act as transcription factor in nucleus – gene expression[2]

RA side effect:
- Oxidative stress
- Less susceptible to get Parkinson's[3]

- RA Tx: homogeneous differentiated SH-SY5Y neurons
- no RA Tx: high proliferation of undifferentiated SH-SY5Y cells[4]

Research Question/Aim:

How does RA treatment affect gene expression in immature neurons?

Group data

- Collected from 3 Thursday PM classes
- 12 genes -> neuronal markers

- RA-affected because increases differentiation
- binds to receptors in promoting region
- regulates gene transcription in early development[5] -> (state of SH-SY5Y cells)

- Down-regulated by differentiation -> cell-cell interaction which stops cellular processes, hence decreasing cell proliferation
- Up-regulated by differentiation -> for cell differentiation

Hypothesis:

RA will change gene expression of different neuronal markers

SNAP25

- Collected from 3 Thursday PM classes
- Presynaptic plasma membrane protein part of SNARE complex
  - **Negatively regulates neurotransmitter release**
  - Negatively regulates intracellular Ca^{2+} from V-gated Ca^{2+} channels[6]
- Normal postsynaptic surface expression of NMDARs[7]

- RA Tx -> levels double[8]
  - Increased differentiation & decrease proliferation -> decrease cellular synaptic pathways

Hypothesis:

RA will increase SNAP25 expression (up-regulation)

METHODOLOGY

Independent variable: RA treatment
Dependent variable: Gene expression level

1. SH-SY5Y treated with 10µM RA (control = no RA) for 72h
2. qPCR steps applied as in Figure 1

- Same treatment done on other sample using RNA sequencing (by technicians)

Baselines:
- qPCR normalized w GAPDH
- RNAseq normalized w TPM

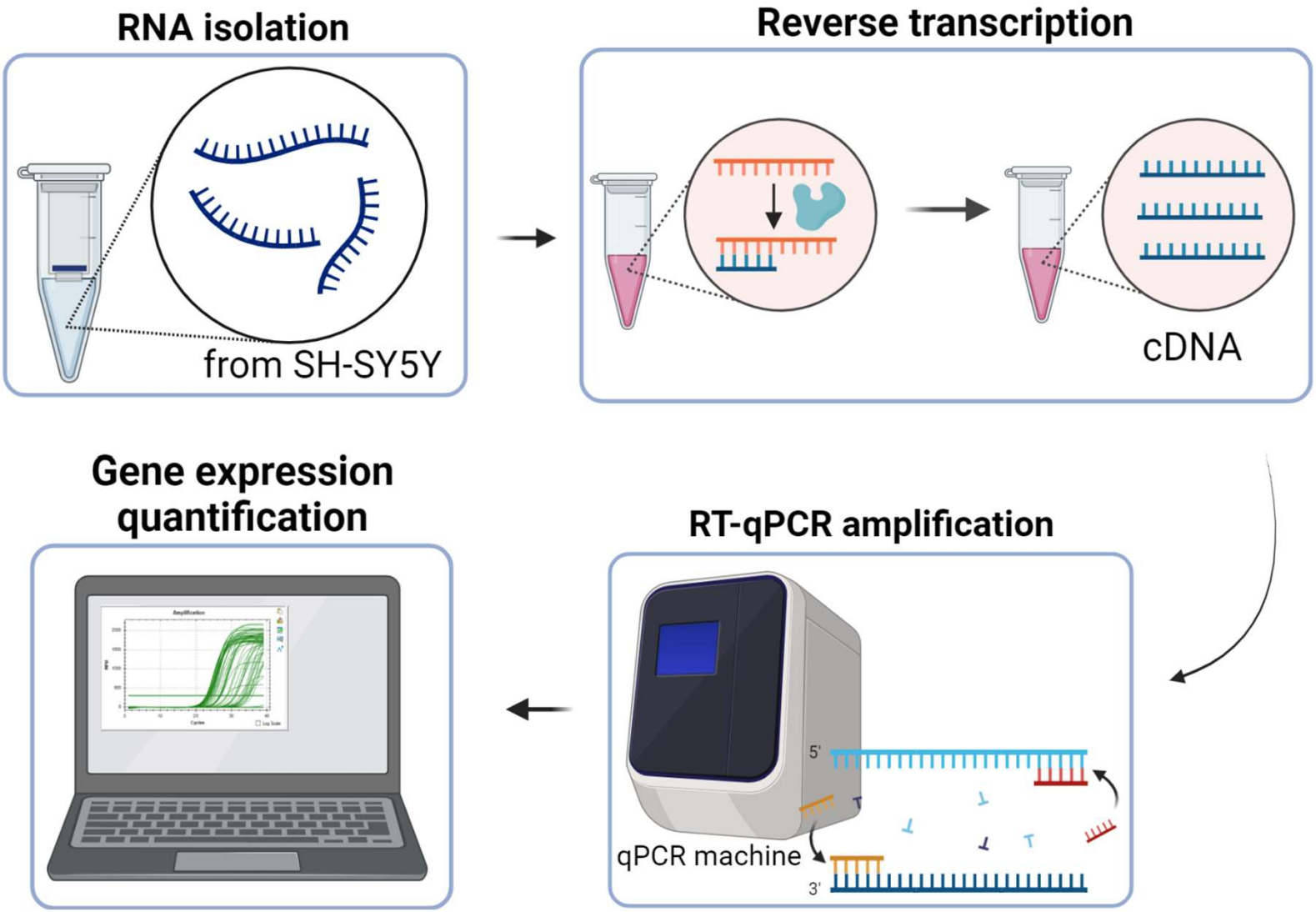
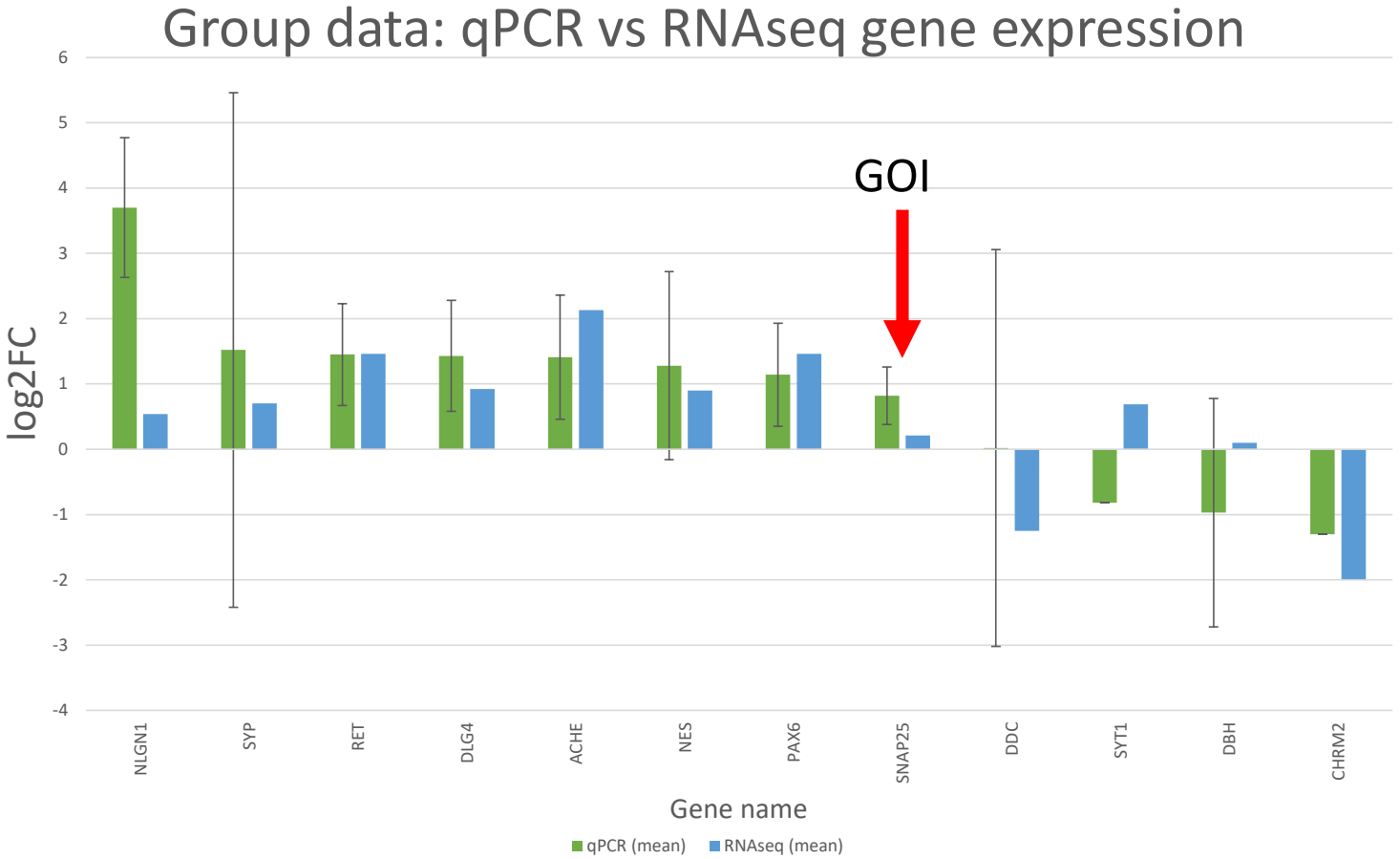


Figure 1: qPCR steps (made with BioRender[9])

RESULTS

Group Data



- qPCR data used SD
- DDC, SYT1, DBH had different directionalities (qPCR vs RNAseq)
  - Up-regulated vs down-regulated

Figure 2: Expression level of 12 neuronal markers after RA Tx for qPCR and RNAseq (made with Excel) – data taken from mean of 3 Thursday PM classes

Figure 3: Up-regulated genes (based on qPCR) – Ontological domains (made by PANTHER[10])

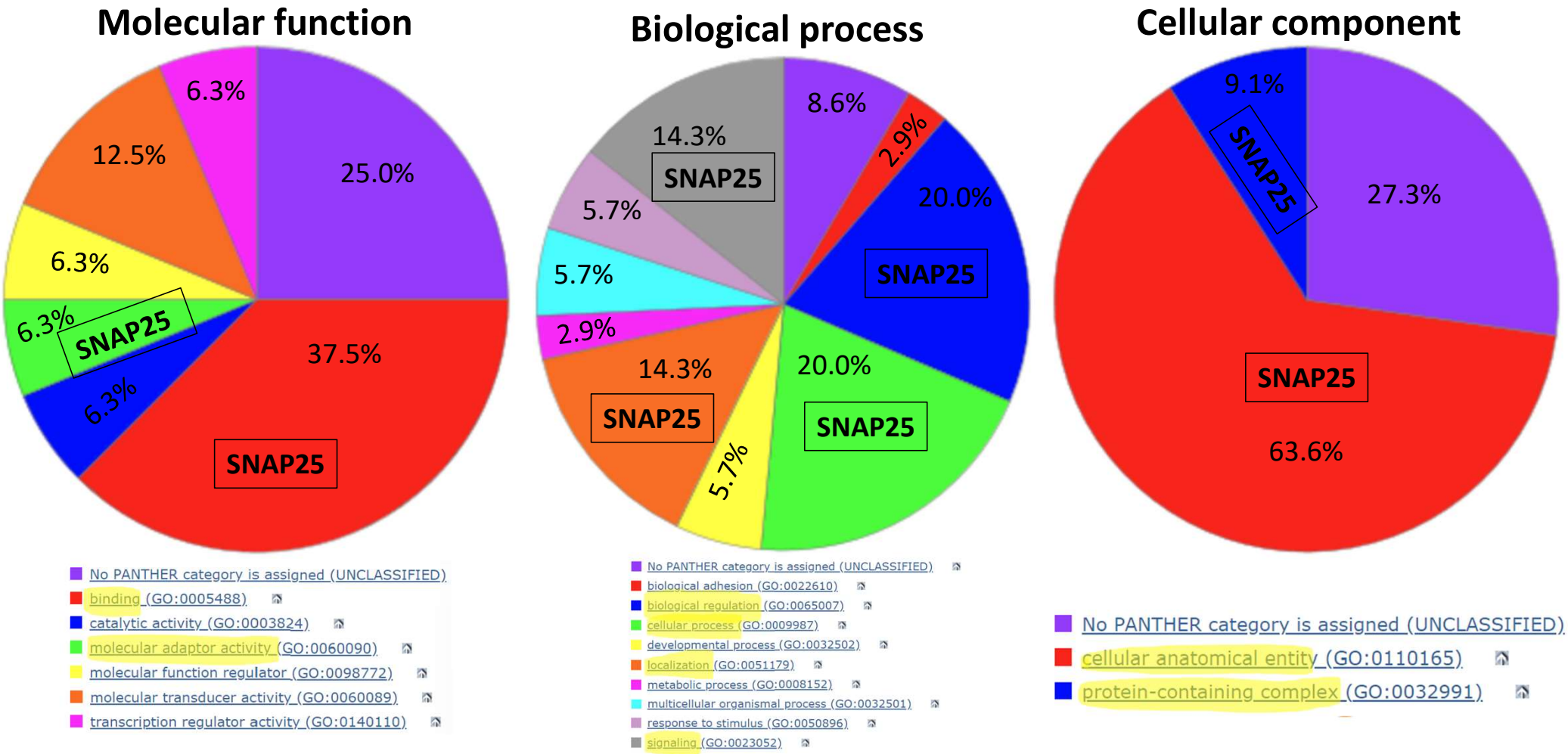
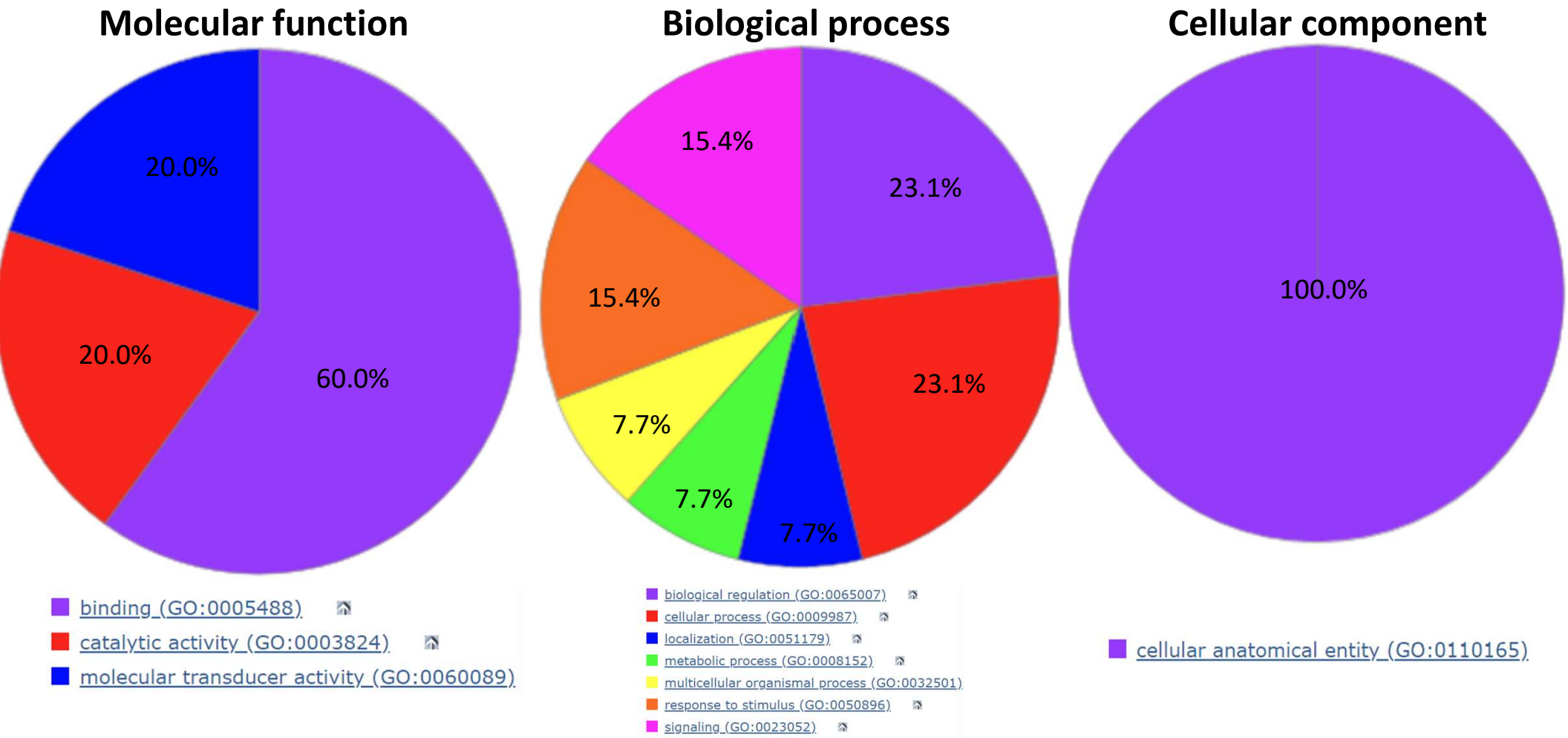
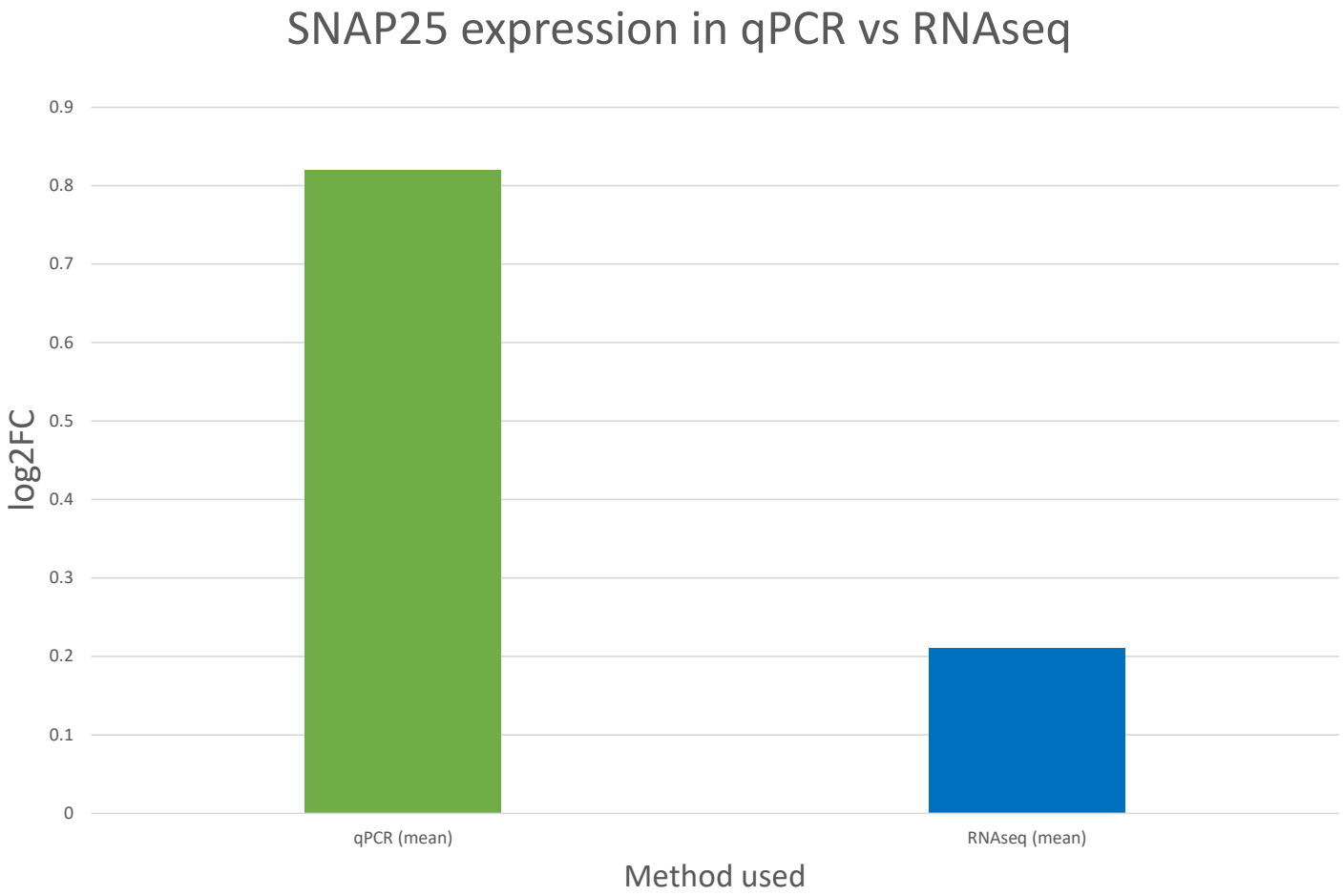


Figure 4: Down-regulated genes (based on qPCR) – Ontological domains (made by PANTHER[10])



SNAP25 - GOI



- Same directionality

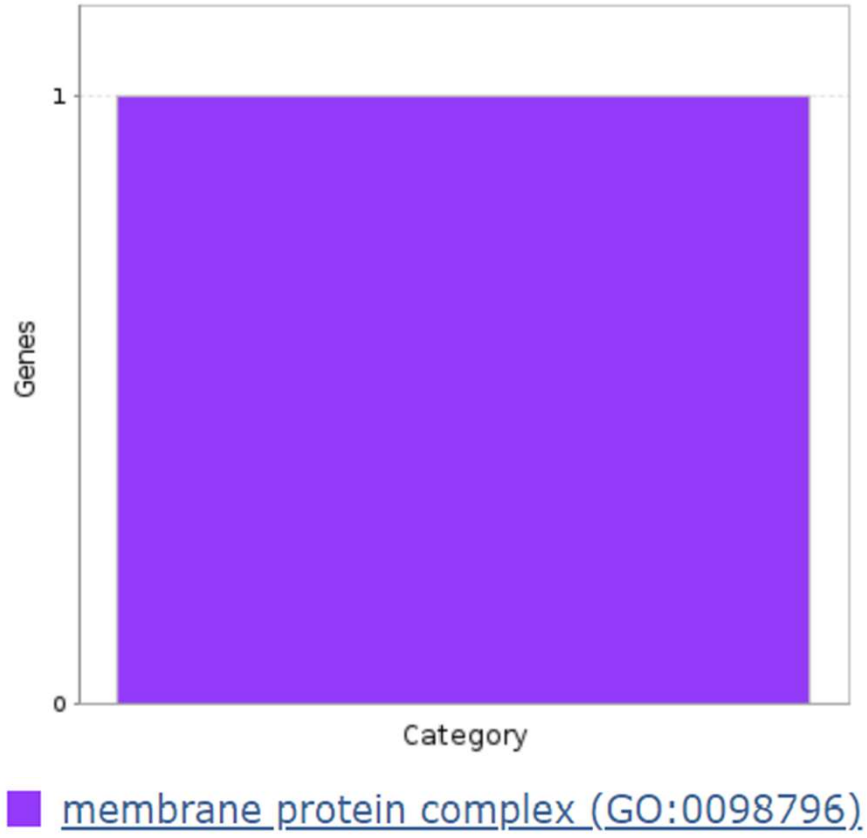
-> **up-regulated**

- qPCR expression level = 0.82 log2FC
  - increase compared to control

Figure 5: SNAP25 expression level after RA Tx for qPCR and RNAseq (made with Excel) - data taken from mean of 3 Thursday PM classes

Ontological subdomain

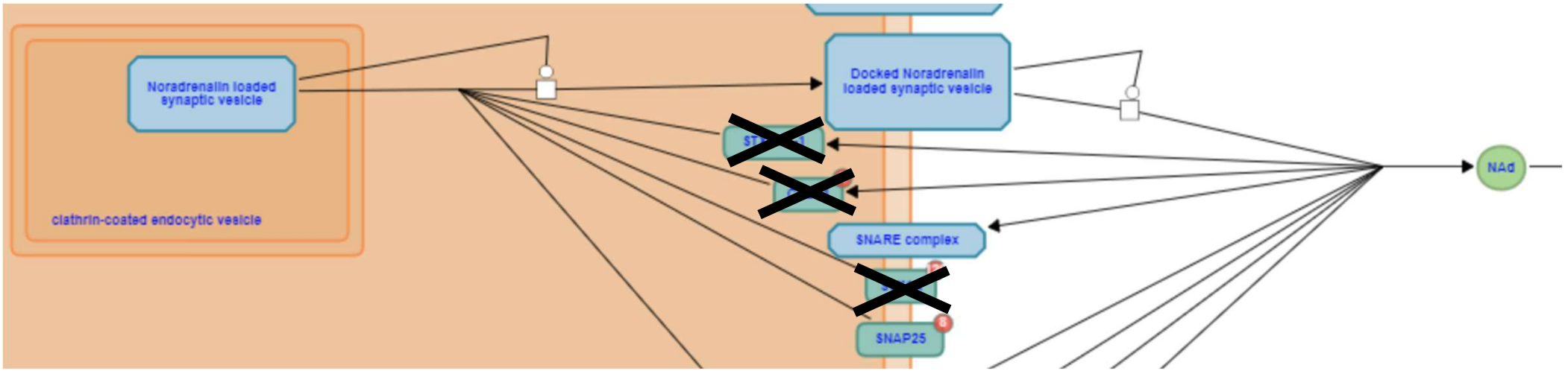
Figure 6: Up-regulated genes **- Protein-containing complex subdomain** of Cellular components (made by PANTHER[10])



- Only SNAP25 (out of up-regulated genes) in protein-containing complex subdomain

→ SNARE complex

Figure 7: Neurotransmitter release cycle pathway involving SNAP25 (from Reactome[11])



- Noradrenaline (NA) vesicle input to SNAP25 (part of SNARE)
- NA gets released and transitioned to extracellular NA
  - Outputs back to SNARE

DISCUSSION - CONCLUSION

- **RA affected gene expression of 12 genes (up/down regulation)**
- **SNAP25 upregulated by RA Tx**

-> both hypotheses were correct

- SNAP25 had low variability (qPCR)
- SNAP25 upregulation -> more neurotransmitters released[8]
- BUT it negatively regulates intracellular Ca2+, so upregulation should decrease neurotransmitter release

- CHRM2, SYT1, DBH were down-regulated (qPCR) -> involved in cell signalling and synaptic activity

- Different directionality for some genes in qPCR vs RNAseq
  -> qPCR not always homogenous amplification[12]
  -> difference amplification efficiencies[13]

Limitations

- RA treatment not long enough
  - neuroblastoma cells have to be differentiated *in vitro* for at least 7 days for SH-SY5Y cells to be used for experiments[14]
- Gene expression change throughout cell differentiation, but was only measured at 72h

Future directions

- Let SH-SY5Y cells differentiae for 1 week
- Samples at different time-points along differentiation process
  - For accurate idea of RA Tx effect on SNAP25

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