Co-expression network analysis of AMPK and autophagy gene products during adipocyte differentiation

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Summary

Background Autophagy is involved in the development and differentiation of many cell types. It is essential for the pre-adipocytes to respond to the differentiation stimuli and may contribute to reorganizing the intracellulum to adapt the morphological and metabolic demands. Although AMPK, an energy sensor, has been associated with autophagy in several cellular processes, how it connects to autophagy during the adipocyte differentiation remains to be investigated.

Methods Here, we studied the interaction between AMPK and autophagy gene products at the mRNA level during adipocyte differentiation using public-access datasets (Figure 1). We used the weighted-gene co-expression analysis [3] to detect and validate multiple interconnected modules of co-expressed genes in a dataset of MDI-induced 3T3-L1 pre-adipocytes (Table 1). Data were obtained, analyzed and visualized using R and Bioconductor packages. The scripts to reproduce this analysis, figures and tables are available here (https://github.com/MahShaaban/aacna)

Results We found that the highly co-expressed gene products of AMPK and autophagy clusters in two, blue and brown, interconnected module (Figure 2 & 3). These modules were found to be highly correlated and significantly over-represented between the differentiation stages of the adipocytes (Figure 4). Moreover, these modules were preserved in three other independent datasets of similar design (Figure 5). Several novel interactions between AMPK and autophagy gene products were identified (Table 2).

Conclusion Together, it is possible that AMPK-autophagy interaction is temporally and locally modulated in response to the differentiation stimuli.

Figures & Tables

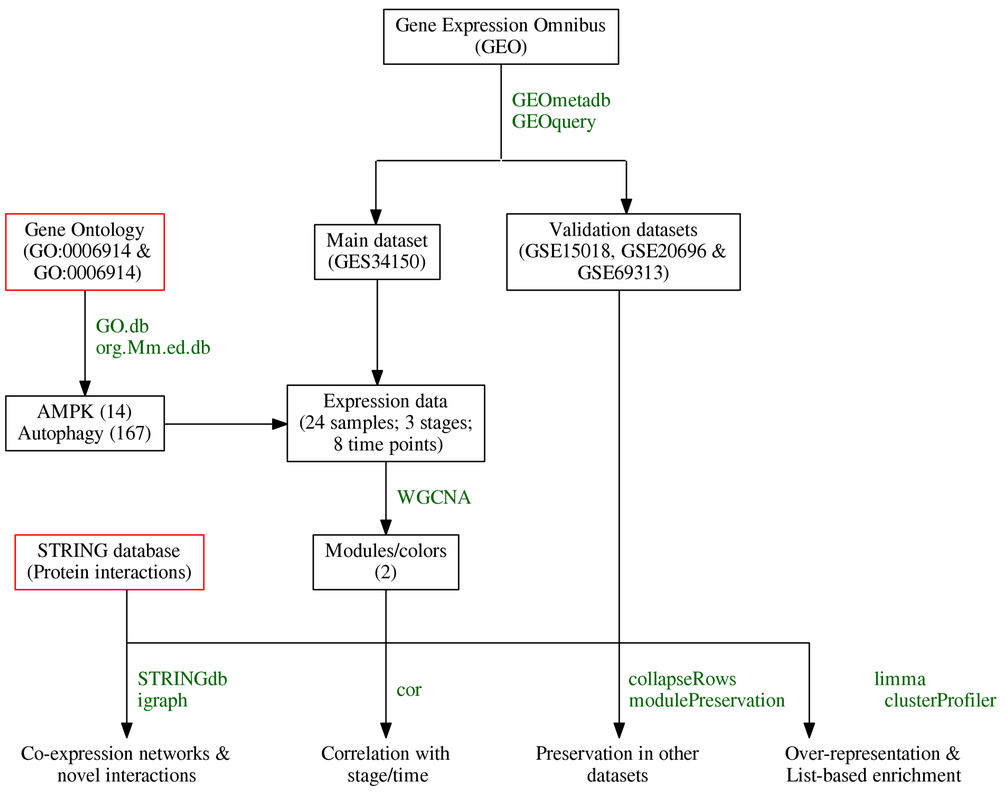

Figure 1: Workflow of the study

Table 1: MDI-induced 3T3-L1 microarrays datasets.

Series ID	Platform ID	Samples	Included	(Contact, year)	Reference
GSE15018	GPL6845	54	18	(Chin, 2009)	[1]
GSE20696	GPL1261	8	8	(Mikkelsen, 2010)	[4]
GSE34150	GPL6885	24	24	(Irmler, 2011)	[2]
GSE69313	GPL6246	48	12	(Renbin, 2015)	[5]

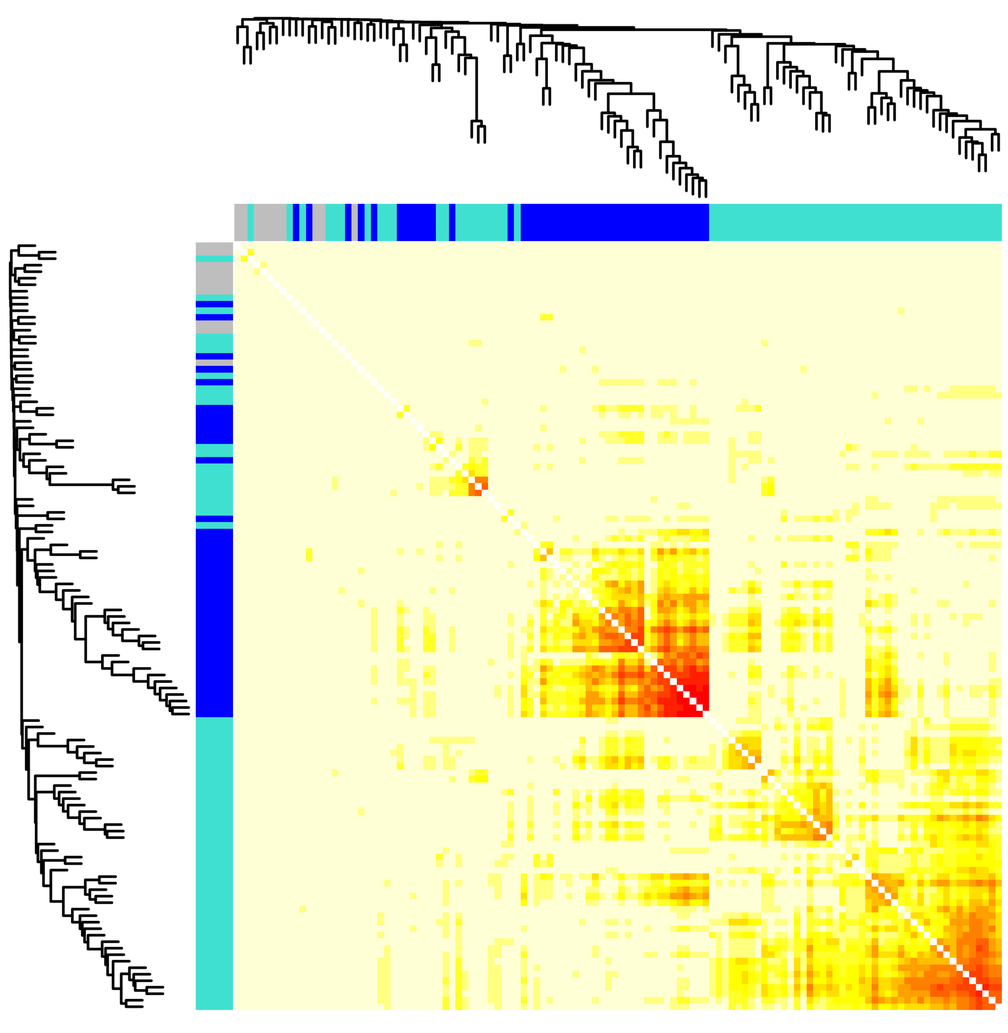

Figure 2: Clustering of AMPK and autophagy genes by their pairwise distances. Pairwise topological overlap matrix (TOM) similarities of AMPK and autophagy genes (n = 181) were calculated from their expression values in the GSE34150 dataset. Distances between each pair of genes were derived as 1 - TOM and shown as color values (small, *red* or large, *yellow*). A hierarchal tree and colored segments of the clusters were shown on the *top* and *side*.

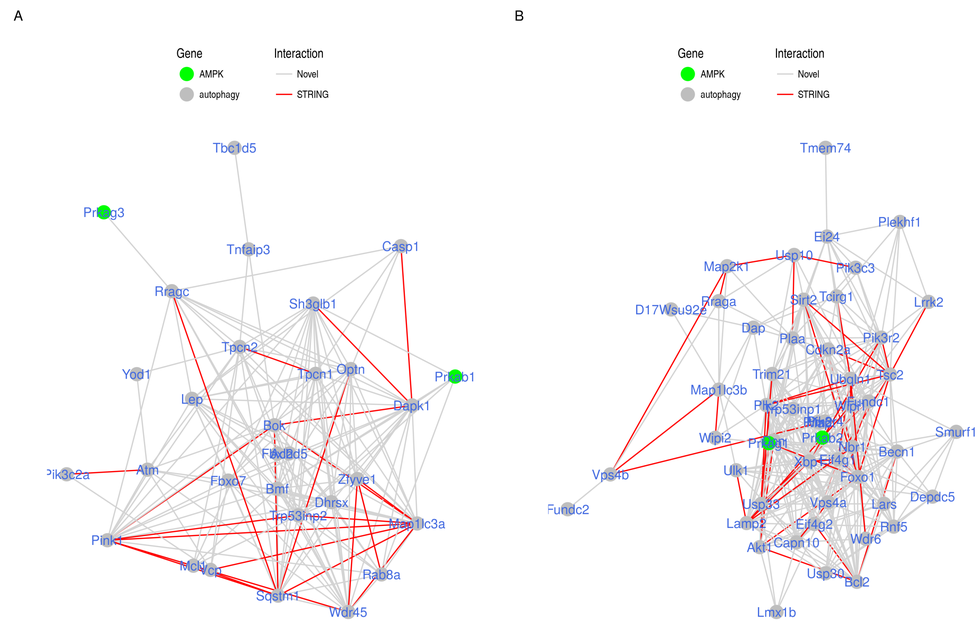

Figure 3: Network representation of the AMPK and autophagy modules. Members of the blue (A) and turquoise (B) modules are shown as a nodes. Each pair of nodes is connected by an edge if the corresponding pairwise topological overlap matrix (TOM) similarity/weight is above the threshold 0.1. Nodes are colored by gene category (AMPK, *green* or autophagy, *gray*). Edges are colored by type of interaction (STRING, *red* or Novel, *gray*).

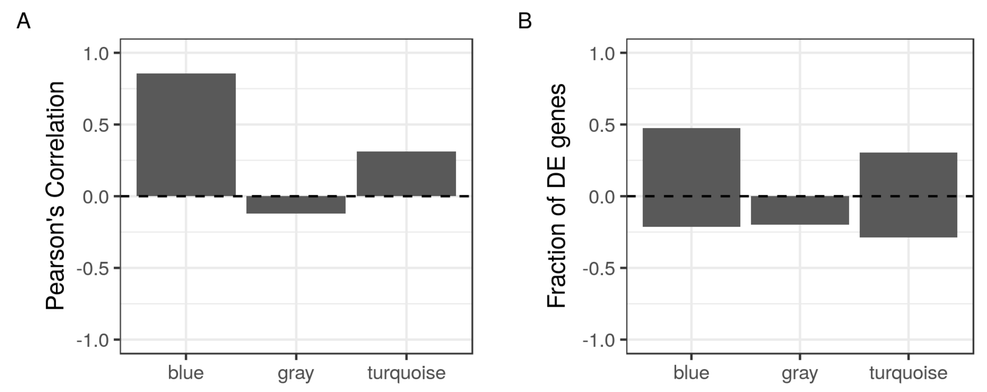

Figure 4: Correlations and over-representation of the detected modules in differentiation stages. The expression values of the members of the detected modules in the GSE34150 dataset (42, blue; 10, gray; and 66, turquoise) were used to calculate two representative summary statistics. (**A**) The first principal component (PC) across samples were correlated to the sample stages using Pearson's correlation (*bars*). (**B**) The fraction of differentially expressed (DE) genes across differentiation stages (*bars*).

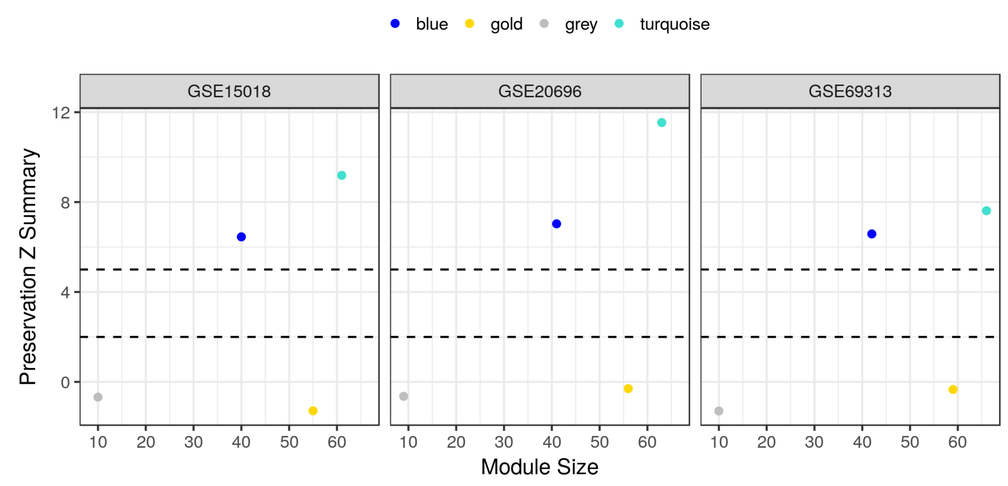

Figure 5: Module preservation Z summary across multiple MDI-induced 3T3-L1 microarrays datasets. The GSE34150 dataset was used to detect the highly co-expressed modules among AMPK and autophagy genes (42, *blue*; 66, *turquoise*; 10, *gray*, unassigned; and 55, *gold*, randomly assigned). The detected modules were used as a reference to calculate several preservation statistics in three independent datasets of similar design (GSE15018, GSE20696 and GSE69313). Z summary statistics and sizes of four modules are shown as colored points.

Table 2: AMPK and autophagy interactions by gene ontology term.

Module/color	Ontology	AMPK	Term	Autophgy
blue	CC	Prkab1	outer membrane	Sh3glb1
	MF		nucleoside binding	Dapk1, Rab8a
			ubiquitin-like protein binding	Trp53inp2
		Prkag3	nucleoside binding	Rragc
turquoise	CC	Prkag1	anchoring junction	Usp33
			extrinsic component of membrane	Becn1, Wipi2
			Flemming body	Vps4a
			intrinsic component of organelle membrane	Fundc1, Lamp2
			midbody	Sirt2, Vps4a
			mitochondrial membrane part	Fundc1
			outer membrane	Bcl2, Capn10, Fundc1
			phosphatidylinositol 3-kinase complex	Becn1
	MF		14-3-3 protein binding	Akt1
			enzyme activator activity	Lars
			kinase regulator activity	Cdkn2a, Dcn
			nucleoside-triphosphatase regulator activity	Lars
			p53 binding	Cdkn2a
			phosphatidylinositol 3-kinase binding	Becn1, Xbp1
			phospholipid binding	Akt1, Wipi2
			protein N-terminus binding	Cdkn2a, Dcn
			ubiquitin-like protein binding	Nbr1, Sirt2
			ubiquitinyl hydrolase activity	Usp33

References

[1] Chin K. *Dataset: A Time Course Analysis of the Effects of Prieurianin in the mouse preadipocytes 3T3-L1 cells*. Toledo, 2010.

[2] M. Horsch et al. *Dataset: Genome-wide expression profiling analysis of a time course of differentiating adipocytes*. Neuherberg, 2015.

[3] P. Langfelder and S. Horvath. "WGCNA: An R package for weighted correlation network analysis". In: *BMC Bioinformatics* 9.1 (Dec. 2008), p. 559.

[4] T. S. Mikkelsen et al. "Comparative epigenomic analysis of murine and human adipogenesis". In: *Cell* 143.1 (2010), pp. 156–169.

[5] M. Zhang et al. *Dataset: Effect of siRNA knock-down of FTO on 3T3-L1 cell differentiation*. Beijing, 2015.