Hyperosmolality in CHO Culture: Effects on Proteome

Nadiya Romanova[1], Louise Schelletter[1], Raimund Hoffrogge[1], and Thomas Noll[1]

[1]Bielefeld University Faculty of Technology

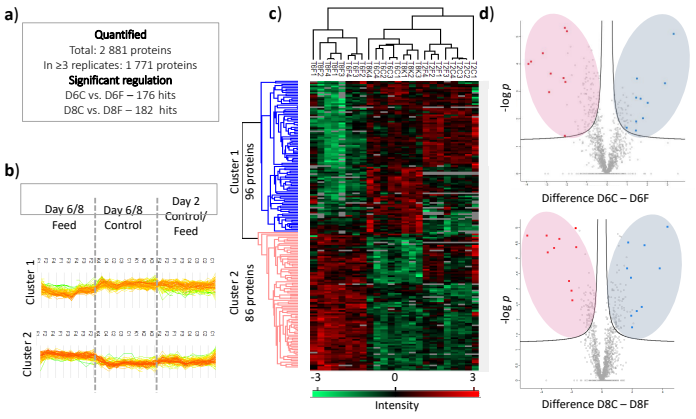
November 12, 2020

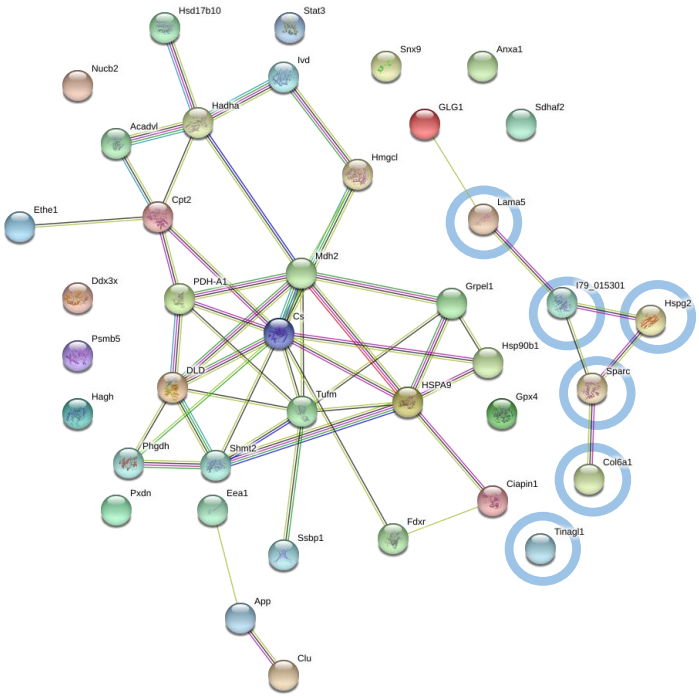
Abstract

Chinese hamster ovary (CHO) is the most commonly used host cell line for therapeutic protein production. Their exposure to highly concentrated feed solution during fed-batch cultivation can cause an unphysiological osmolality increase (>300 mOsm/kg) affecting cell physiology, morphology, and proteome. In a companion article "Hyperosmolality in CHO Culture: Effects on Cellular Behavior and Morphology" we show that hyperosmolalities of up to 545 mOsm/kg force cells to ablate proliferation and gradually increase their volume, almost triplicating it. CHO cells also exhibit a significant hyperosmolality-dependent mitochondrial activity increase. To get a deeper insight into molecular mechanisms involved in these processes, we performed a comparative quantitative label-free proteome study of hyperosmolality-exposed vs. control CHO cells. Our analysis revealed key differentially expressed proteins mediating mitochondrial activation, oxidative stress amelioration, and cell cycle progression. We also discovered a previously unknown strong regulation of proteins altering cell membrane rigidity and permeability. Among others, we detected three members of septins, filamentous proteins forming diffusion barriers in the cell, to be highly upregulated in response to hyperosmolality. Taken together, our observations correlate well with the recent CHO-based fluxome and transcriptome studies and expose new unknown targets involved in response to hyperosmotic pressure in mammalian cells.

Hosted file

`Hyperosmolality in CHO culture - Proteome.pdf` available at https://authorea.com/users/375355/articles/492610-hyperosmolality-in-cho-culture-effects-on-proteome





Node 1	Node 2	Node 1 annotation	Node 2 annotation	Confidence score
Mdh2	Cs	Malate dehydrohenase 2	Cytrate synthase	0.996
PDH-A1	DLD	Pyruvate dehydrohenase E1	Dihydrolipoyl dehydrohenase, m	0.991
Acadvl	Hadha	VLC-specific acyl-CoA dehydrogenase	Trifunctional enzyme subunit alpha	0.967
Grpel1	HSPA9	GrpE protein homolog 1	Stress-70 protein, mitochondrial	0.964
Acadvl	Cpt2	VLC-specific acyl-CoA dehydrogenase	Carnitine O-palmitoyltransferase 2	0.961
Ivd	Hadha	Isovaleryl-CoA dehydrogenase	Trifunctional enzyme subunit alpha	0.954
Hsd17b10	Hadha	3-hydroxyacyl-CoA dehydrogenase	Trifunctional enzyme subunit alpha	0.931
Mdh2	DLD	Malate dehydrohenase 2	Dihydrolipoyl dehydrogenase	0.89
Hmgcl	Cs	Hydroxymethylglutaryl-CoA lyase	Cytrate synthase	0.877
I79_015301	Hspg2	Nidogen 1.2	Heparan sulfate core protein	0.867
Tufm	HSPA9	Mitochondrial elongation factor Tu	Stress-70 protein, mitochondrial	0.859
Col6a1	Sparc	Collagen alpha-1(VI) chain	Kazal-like domain-containing protein	0.84
Tufm	Mdh2	Mitochondrial elongation factor Tu	Malate dehydrohenase 2	0.828
Shmt2	DLD	Serine hydroxymethyltransferase	Dihydrolipoyl dehydrohenase, m	0.823
Tufm	DLD	Mitochondrial elongation factor Tu	Dihydrolipoyl dehydrohenase, m	0.776
Tufm	Grpel1	Mitochondrial elongation factor Tu	GrpE protein homolog 1	0.768
I79_015301	Sparc	Nidogen 1.2	Kazal-like domain-containing protein	0.76
App	Clu	Amyloid-beta A4 protein	Clusterin	0.736
Hsp90b1	HSPA9	HSP90, beta	Stress-70 protein, mitochondrial	0.734
Mdh2	Hmgcl	Malate dehydrohenase 2	Hydroxymethylglutaryl-CoA lyase	0.711
Cpt2	PDH-A1	Carnitine O-palmitoyltransferase 2	Pyruvate dehydrogenase E1, subunit α	0.703
Shmt2	Tufm	Serine hydroxymethyltransferase	Mitochondrial elongation factor Tu	0.692