Comparative proteomic analysis of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) of different origin

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Background and Objective

Induced pluripotent stem cells (iPSC) can be obtained from somatic cells using ectopic expression of 4 basic reprogramming factors: **OCT4** (also known as POU5F1), **SOX2**, Krüppel-like factor 4 (**KLF4**) and MYC (also known as **c-MYC**). Somatic cells pass through the early, middle and late phases of reprogramming towards iPSCs.

The generation of human iPSCs (hiPSCs) from adult somatic cells holds important scientific and clinical implications. While many studies have characterized and compared gene expression profiles of hiPSCs and human embryonic stem cells (hESCs), only few have dealt with their proteomes. iPSCs have been found to be highly similar to ESCs, but their gene expression profiles differ from ESCs: iPSCs may not completely silence the expression pattern of somatic cells, however, ESC signature genes are expressed at higher levels in iPSCs after extended culture.

There are 19 clinical trials now registered using iPSC technology. There is a strong interest on iPSCs therapeutic potential ranging from autism and bipolar disorder to traumatic spinal cord injury.

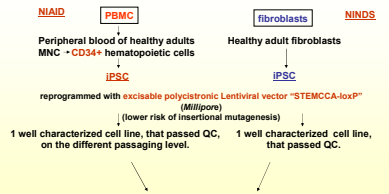
The current proteomic study aims to answer several questions:

Does the somatic origin, reprogramming method, culture condition, passage number affect different iPSC lines?

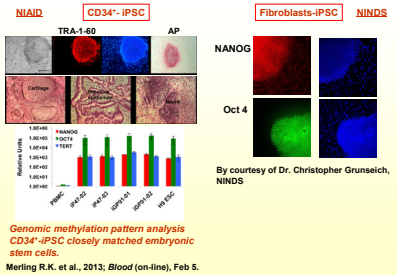
What are the differences between hiPSCs and hESCs on the molecular and functional levels?

Materials and Methods

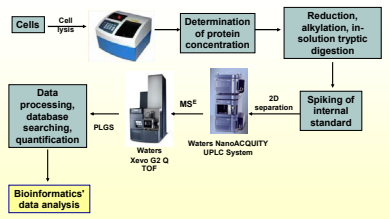
Study design:



Quality control of both iPSC lines has been performed.



Proteomics workflow:



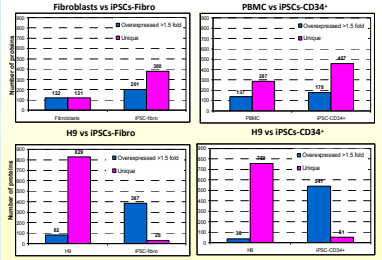
Results

Number of proteins identified from different cell lines

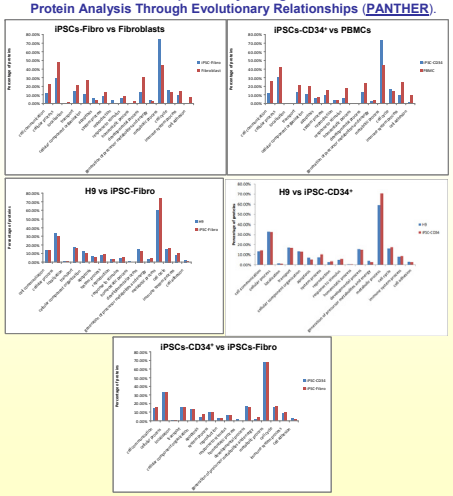
Method: 20fraction 2D-LC/MS^E

	Sample	Replicates	No. proteins identified
ESC	H9 (P. 34)	run-1	2668
		run-2	3155
		run-3	2894
		Combined	3784
		Overlap	2402
iPSC	iPSC-fibro (P. 28)	run-1	1810
		run-2	1910
		run-3	1430
		Combined	2383
		Overlap	1051
	iPSC-CD34 (P. 53)	run-1	2327
		run-2	2141
		run-3	1541
		Combined	2803
		Overlap	1341
Primary somatic cells	Fibroblast	run-1	1628
		run-2	923
		run-3	1501
		Combined	1876
		Overlap	880
	PBMC	run-1	1664
		run-2	1999
		run-3	1427
		Combined	2270
		Overlap	967

Differentially expressed proteins



General Comparison by Biological Process. Protein Analysis Through Evolutionary Relationships (PANTHER).



Ingenuity Pathway Analysis (IPA) of differentially expressed proteins

iPSC-Fibro vs. fibroblasts

Number of proteins

Rank	Top network	score	iPSC-Fibro > fibroblasts	iPSC-Fibro < fibroblasts
1	RNA Post-Transcriptional Modification, Molecular Transport, RNA Trafficking	58	33	0
2	Cancer, Hematological Disease, RNA Post-Transcriptional Modification	50	28	2
3	Cell Morphology, Cellular Function and Maintenance, DNA Replication, Recombination, and Repair	50	24	4

iPSC-CD34+ vs. PBMC

Number of proteins

Rank	Top network	score	iPSC-CD34> PBMC	iPSCCD34< PBMC
1	Cell Cycle, DNA Replication, Recombination, and Repair, Cellular Response to Therapeutics	54	25	7
2	RNA Post-Transcriptional Modification, Neurological Disease, Molecular Transport	51	30	1
3	Cancer, Hematological Disease, RNA Post-Transcriptional Modification	51	31	0

iPSC-CD34+ vs. H9

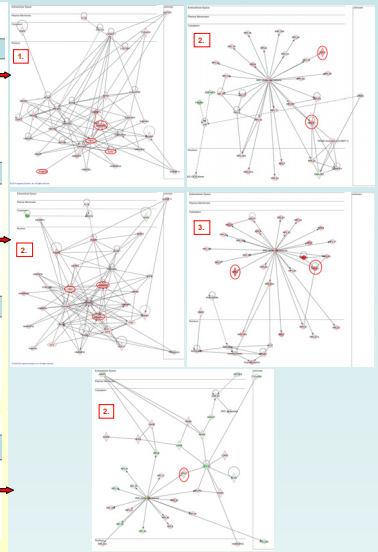
Number of proteins

Rank	Top network	score	iPSC-CD34> H9	iPSC-CD34< H9
1	RNA Post-Transcriptional Modification, Molecular Transport, RNA Trafficking	52	15	18
2	Developmental Disorder, Hematological Disease, Hereditary Disorder	49	21	11
3	RNA Post-Transcriptional Modification, Protein Synthesis, Developmental Disorder	49	14	18

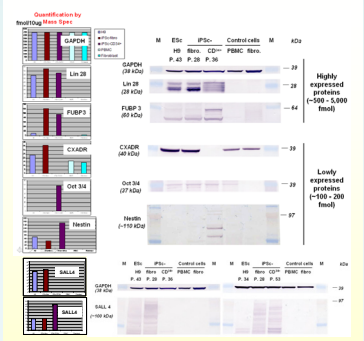
iPSC-Fibro vs. H9

Number of proteins

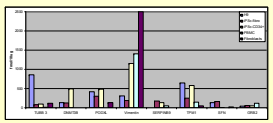
Rank	Top network	score	iPSC-Fibro > H9	iPSC-Fibro < H9
1	Developmental Disorder, Hematological Disease, Hereditary Disorder	53	21	12
2	Cancer, Hematological Disease, Gastrointestinal Disease	51	17	15
3	RNA Post-Transcriptional Modification, Dermatological Diseases and Conditions, Infectious Disease	50	11	21



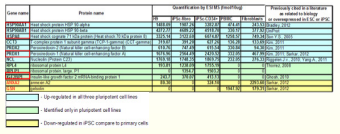
Comparison of quantification by ESI MS-MS and Western blot results



Previously proposed markers of pluripotency identified by ESI MS-MS



12 novel potential markers for iPSC were proposed and some were confirmed by Western-blot analysis

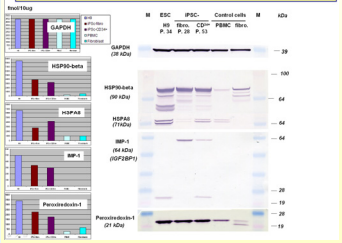


Up-regulated in all three pluripotent cell lines

Identified only in pluripotent cell lines

Down-regulated in iPSC compare to primary cells

Verification of the novel potential markers for iPSC by Western-blots analysis



Conclusions

1) **Considerable differences in protein expression profiles was observed between the iPSCs and parental somatic cells. The major differences were revealed in:**
RNA Post-Transcriptional Modification, RNA Trafficking, Molecular Transport,
Protein synthesis, Ribosomal complex,
Cell Morphology,
Cell cycle,
DNA Replication, Recombination, and Repair.

2) **The protein expression profiles of iPSCs and ESCs were different. The biological functions affected were very similar to those affected in comparison with primary cells.**

3) **The following pluripotency markers were quantified by ESI MS-MS in both ESCs and iPSCs: SALL4; Lin28; FUBP3; Oct3/4; Nestin; TUBB3; DNMT3B; PODXL; Serpin B9; TPM1 and SFN (14-3-3 protein Sigma).**

4) **12 novel iPSC functional markers were proposed and some were confirmed by Western-blot: HSP90-alpha; HSP90-beta; HSPA8; IMP1; Peroxiredoxin-1.**

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