Cordycepin maintained the pluripotency of ES and iPS cells by activating ECM and Jak2-Stat3 signaling pathway

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ABSTRACT

Embryonic stem (ES) cells and induced pluripotnet stem (iPS) cells are both pluripotent cells. Maintaining the pluripotency of ES and iPS cell cultures required leukemia inhibitory factor (LIF). However, LIF is an expensive reagent. The aim of this study was to find out a pure compound extract from Chinese herbal medicine could replace LIF and maintain ES and iPS cells pluripotency. We determined that *Cordyceps militaris* from 15 candidates could upregulated Oct4 and Sox2 gene expression levels in MEF cells. We also demonstrated the pure compound of *Cordyceps militaris*, Cordycepin, specially in 10μM could upregulated Oct4 and Sox2 gene expression levels in ES and iPS cells, too. Then we used ES and iPS cells treated with different concentrations of Cordycepin (replaced the LIF in the culture medium) to test whether it was useful to maintain the pluripotency of ES and iPS cells. The results indicated higher expression levels of several stem cell markers in Cordycepin-treated ES and iPS cells that compared with controls (not containing LIF), including alkaline phosphatase, SSEA1, and Nanog. Embryonic body formation and differentiation confirmed that Cordycepin replaced medium culture was capable of maintain ES cell pluripotency. Microarray analysis showed that the top three altered pathway were ECM, calcium, and Jak-Stat signaling pathway. We subsequently determined that phosphorylated Src, phosphorylated Jak2, and phosphorylated Stat3 protein levels increased following Cordycepin treatment. The gene expression of integrin αVβ5 and cytokines associated with Jak2/Stat3 signaling pathway were upregulated, too. In conclusion, our data indicated that cordycepin could maintain the pluripotency of stem cells through both of ECM and Jak2/Stat3 signaling pathway.

RESULTS

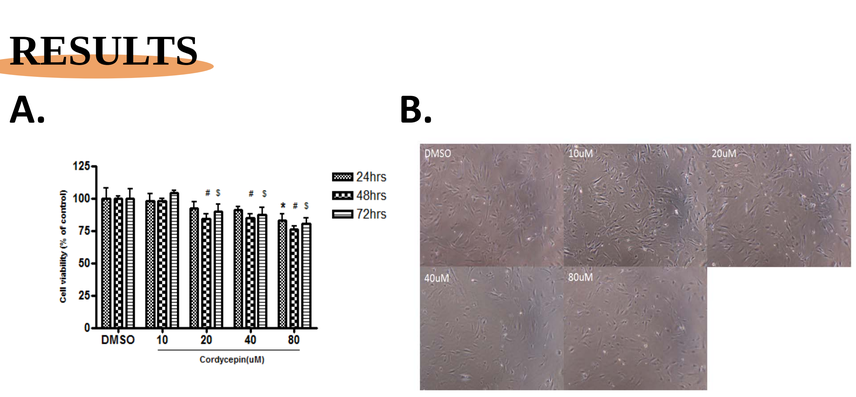

Figure 1. A) MTT assay for the MEF cells that treated with different concentration of Cordycepin. B) Microscopy of MEF cells that treated with different concentration of Cordycepin.

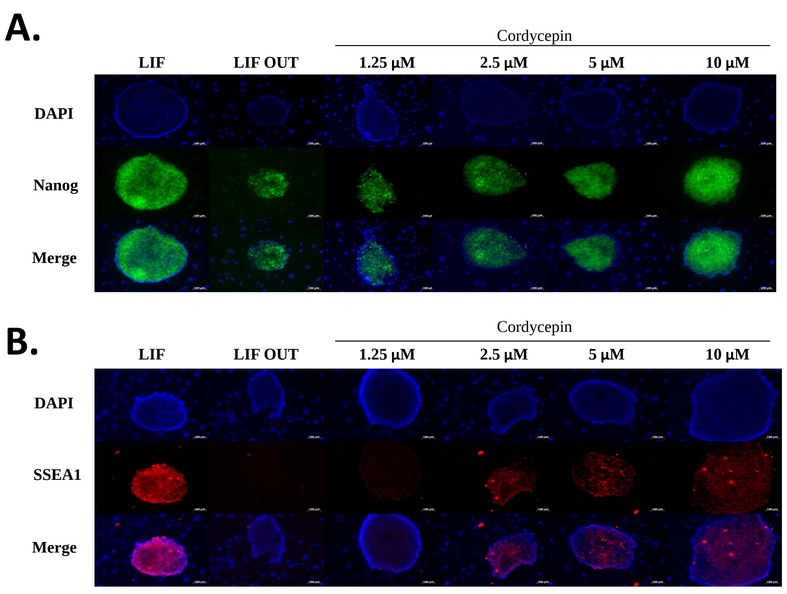

Figure 2. A) Immunocytochemistry of Nanog in ES cells treated with different concentration of Cordycepin. B) Immunocytochemistry of SSEA1 in ES cells treated with different concentration of Cordycepin.

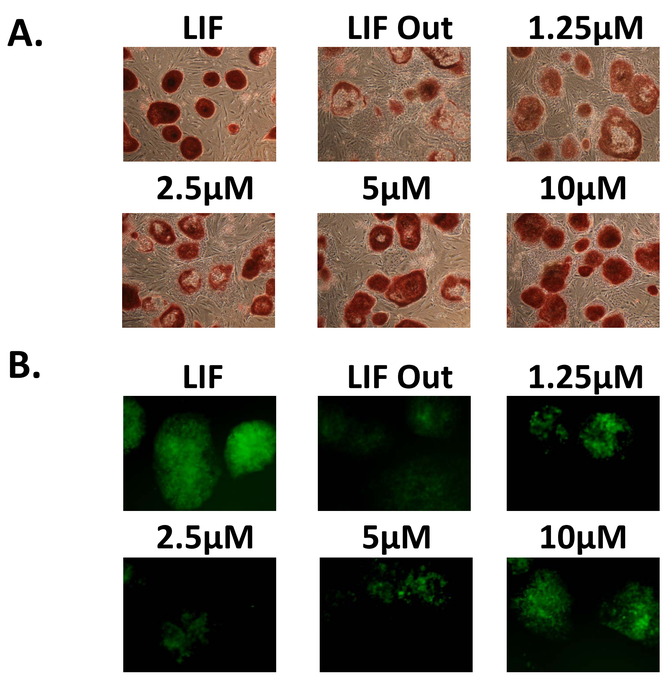

Figure 3. A) Microscopy of alkaline phosphatase staining for the iPS cells that treated with different concentration of Cordycepin. B) Microscopy of Nanog-GFP expression levels for the iPS cells that treated with different concentration of Cordycepin.

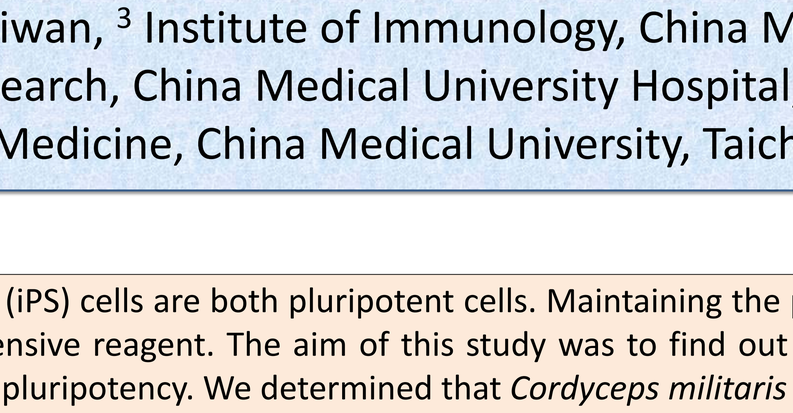

Figure 4. A) Immunocytochemistry of Nanog in iPS cells treated with different concentration of Cordycepin. B) Immunocytochemistry of SSEA1 in iPS cells treated with different concentration of Cordycepin.

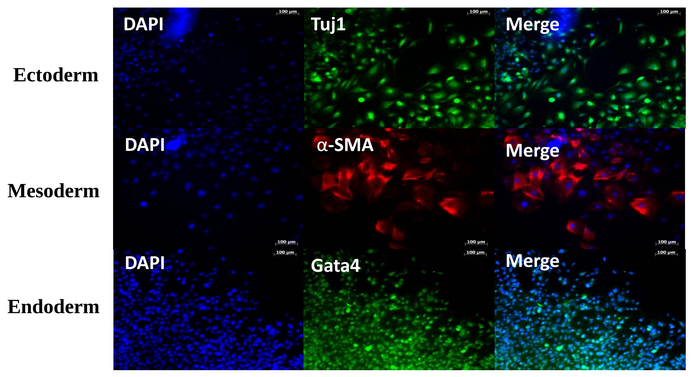

Figure 5. Embryoid body-mediated differentiation of ES cells passage four times in Cordycepin containing medium (replace LIF). Immunofluorescent staining of Tuj1 (ectoderm marker), α-smooth muscle actin (mesoderm marker), Gata4 (endoderm marker)**.** Nuclei were stained with DAPI (blue).

Table1.Numbers of significantly deregulated genes with known biological functions classified according to KEGG and Babelomics database

Function / Pathway	Cordycepin 10(μM) I	D	n	%
Methylation (n=0)				
Signal transduction				
ECM-receptor interaction	4	17	21/37	56.76
Jak-Stat signaling pathway	6	14	20/46	43.48
Calcium signaling pathway	4	18	22/51	43.14
VEGF signaling pathway	1	3	4/12	33.33
PPAR signaling pathway	1	7	8/29	27.59
Insulin signaling pathway	12	4	16/59	27.12
TGF-beta signaling pathway	2	8	10/40	25.00
Wnt signaling pathway	4	9	13/52	25.00
MAPK signaling	4	18	22/97	22.68
Cell proliferation				
Cell communication	8	11	19/33	57.58
Cell cycle	6	9	15/67	22.39
Metabolism				
Amino acid metabolism	3	3	6/21	28.57
Lipid metabolism	7	7	14/69	20.29
Cell adhesion				
Cell adhesion molecules	9	18	27/55	49.09
Focal adhesion	5	26	31/78	39.74
Tight junction	9	8	17/51	33.33
Apoptosis	0	14	14/41	34.15

I: number of upregulated genes ; D:number of downregulated genes

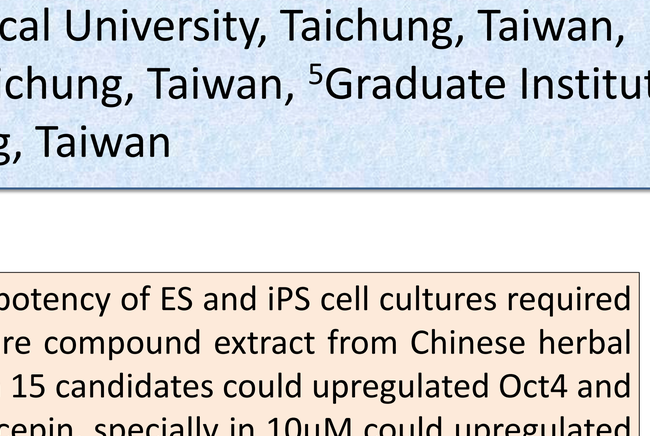

Figure 6. A)Real-time PCR analysis of cytokine genes expression levels involved in the Jak2-Stat3 pathway. B) Phosphorylation level of Jak2 and Stat3 in mES cells was analyzed by Western blotting using antibodies to Jak2, Stat3, and their phosphorylated forms.

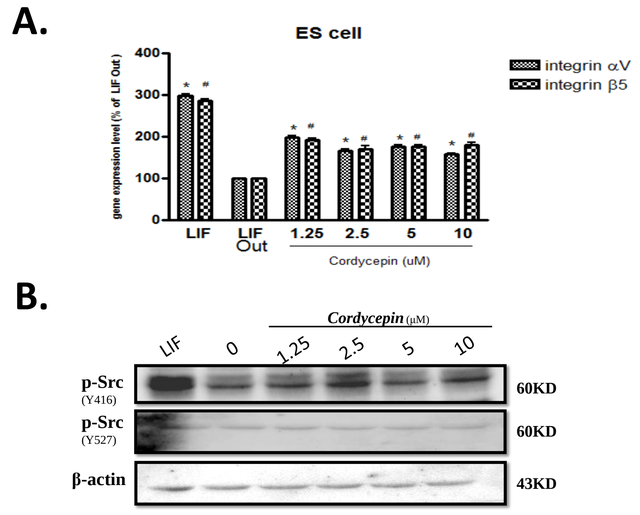

Figure 7. A) Real-time PCR analysis of integrin αVβ5 genes expression levels in ES cells. B) Phosphorylation level of Src in mES cells was analyzed by Western blotting using antibodies to p-Src (Y416), and p-Src (Y527) phosphorylated forms.

Conclusions

In conclusion, our data indicated that Cordycepin could maintain the pluripotency of stem cells through both of ECM and Jak2/Stat3 signaling pathway.