Cell Surface and Functional Features of Cortical Bone Stem Cells

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Research

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Abstract

Background: The newly established mouse cortical bone–derived stem cells (mCBSCs) are unique stem cells compared with mouse mesenchymal stem cells (mMSCs), and can improve cardiac function after myocardial infarction. However, the mCBSCs' characterizations including their stem cell features, non-cardiac therapeutic potential, and cell surface features have not been fully understood. In this study, we examined stem cell features, cell surface glycan profiles, and cell functional features in mCBSCs compared to the bone marrow-derived mMSCs.

Methods: The stem cell features were compared between mCBSCs and mMSCs by immunoblotting of stem cell markers, self-renewal assay, and multilineage differentiation. The cell surface glycan profiles were examined by lectin array analysis and fluorescence-activated cell sorting analysis using lectins. The production of transforming growth factor (TGF)-β1 from mCBSCs were examined by ELISA. The effects of TGF-β1 released from mCBSCs on self-migration and on activation of fibroblast were examined by migration assay and immunocytostaining, respectively.

Results: The stem cell feature, including the self-renewing ability in mCBSCs was higher than that in mMSCs. In contrast, the differentiation ability of mCBSCs was limited to the chondrogenic lineage among three types of cells (adipocyte, osteoblast, chondrocyte). The cell surface glycan profiles revealed that α2-6sialic acid is expressed at very low levels on the cell surface of mCBSCs compared with that on mMSCs. Additionally, the lactosamine (Galβ1-4GlcNAc)-structure, poly lactosamine- or poly *N*-acetylglucosamine-structure, and α2-3sialic acid on both *N*- and *O*-glycans are more highly expressed in mCBSCs compared with mMSCs. Furthermore, these highly expressed glycans were increased with cellular aging of mCBSCs. We found that TGF-β1 was released from mCBSCs and the released TGF-β1 contributed to the self-migration of mCBSCs and activation of fibroblasts.

Conclusions: These results reveal the differences between mCBSCs and mMSCs, and it is proposed that there is the potential use of mCBSCs for infarct healing and wound healing.

Full Text

This preprint is available for download as a PDF.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

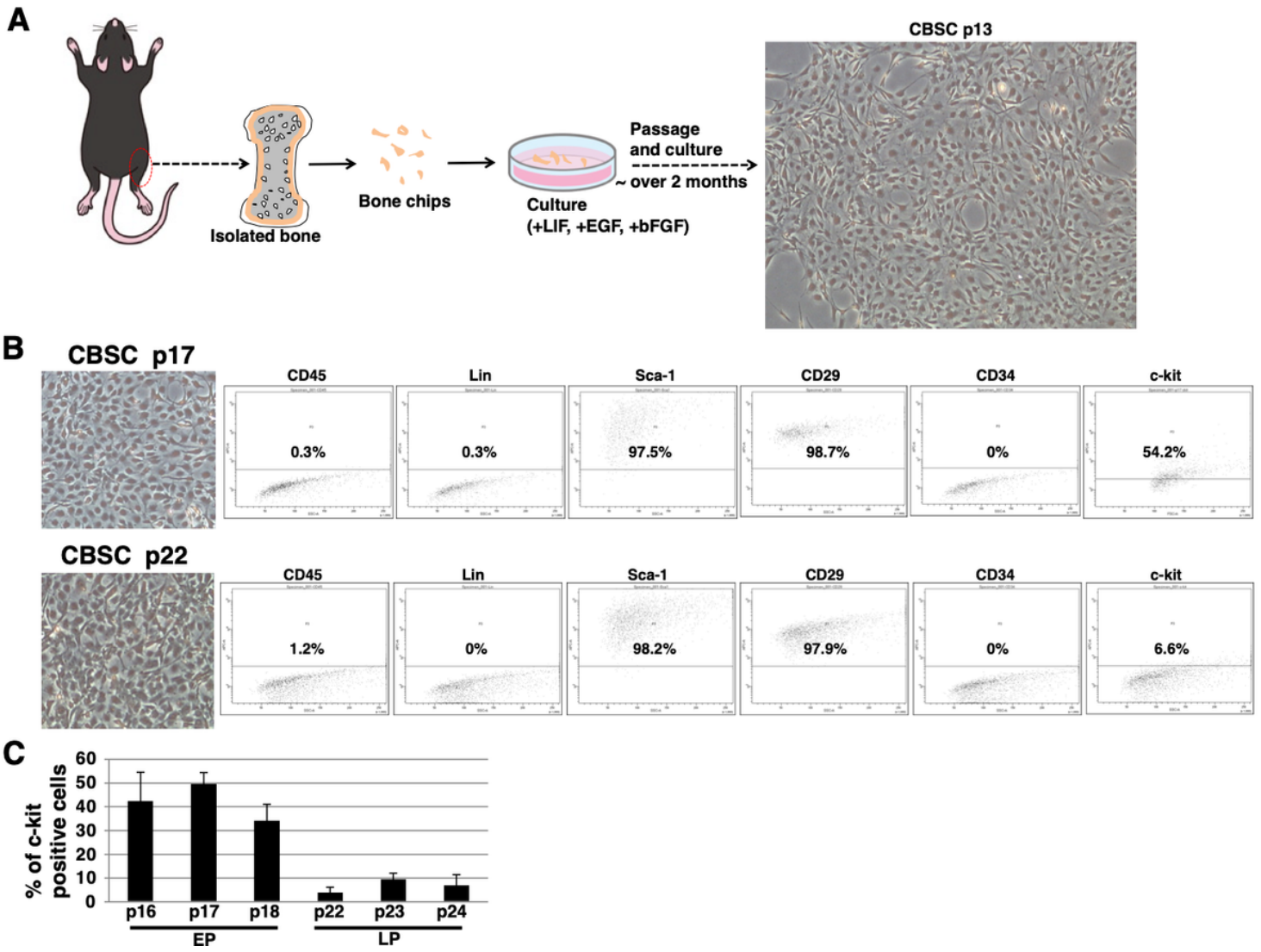

Figure 1

Isolation of mCBSCs. a Scheme of isolation and culture of mCBSCs. The bone biopsy was isolated from one limb. Then, bone chips were prepared and cultured with mCBSCs culture media. About 2 months after passage and culture, mCBSCs was observed. b FACS analysis of CD45, Lin, Sca- 1, CD29, CD34, and c-kit in mCBSCs (p17 and p22). Three independent experiments were performed and representative results are shown. c Percent of c-kit positive cells in mCBSCs (p16-p18 and p22- p24) is shown. The values shown are the means ± SD from three independent experiments. Abbreviations: mCBSC, mouse cortical bone derived stem cell; EP, early passage; LP, late passage.

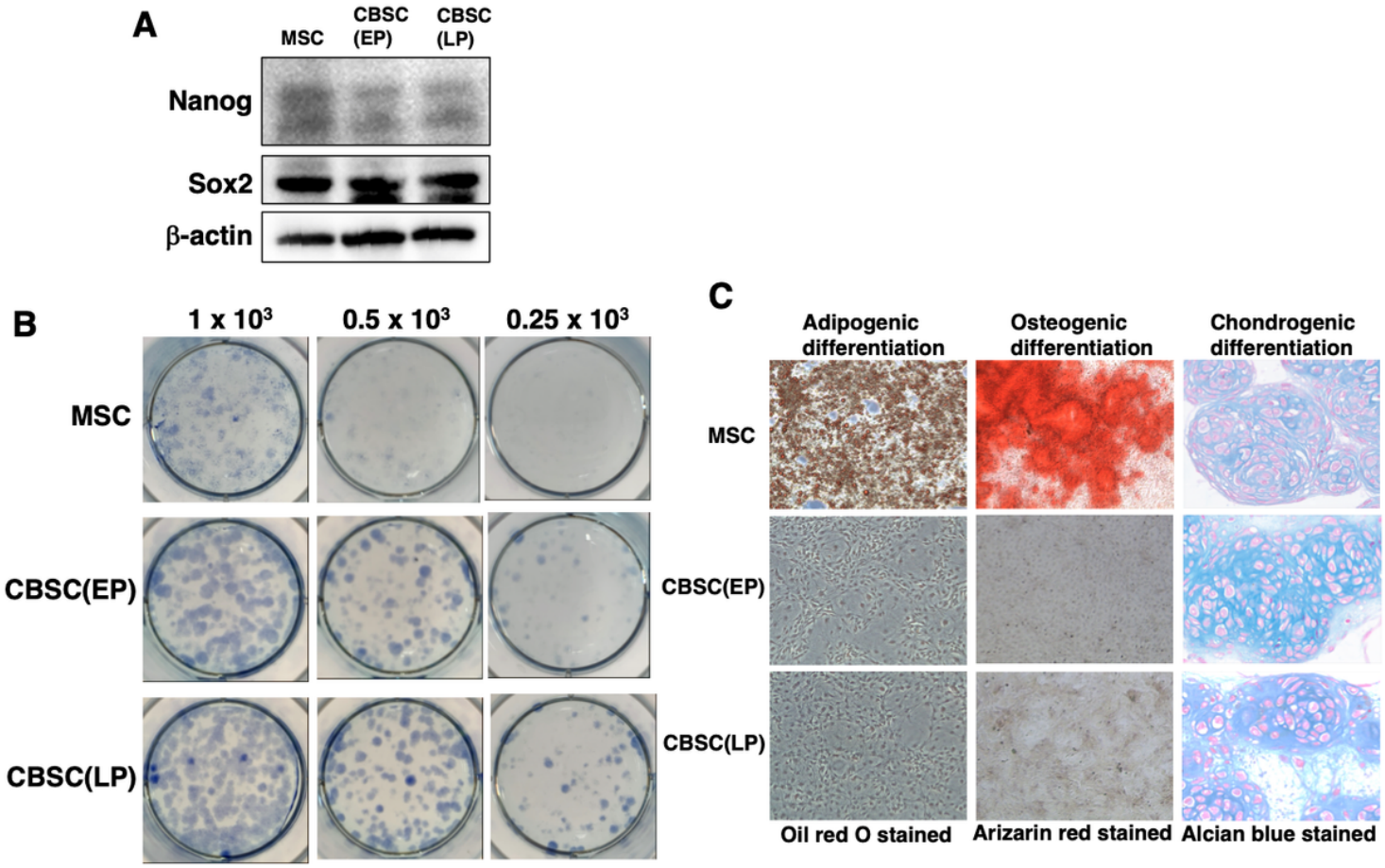

Figure 2

Stemness features of mCBSCs. a Western blot analysis of Nanog, Sox2, and β-actin (loading control) in mMSCs and mCBSCs (EP and LP). b Colony forming assay in mMSCs and mCBSCs (EP and LP). Cells were plated at low density (0.25 x 103 cells, 0.5 x 103 cells, or 1 x 103 cells/well) and cultured. After 7 days, cells were stained and photos were captured. c Adipogenic, Osteogenic, or Chondrogenic differentiation was induced in mMSCs and mCBSCs (EP and LP). Representative images are shown. Abbreviations: mMSC, mouse mesenchymal stem cell; mCBSC, mouse cortical bone derived stem cell; EP, early passage; LP, late passage.

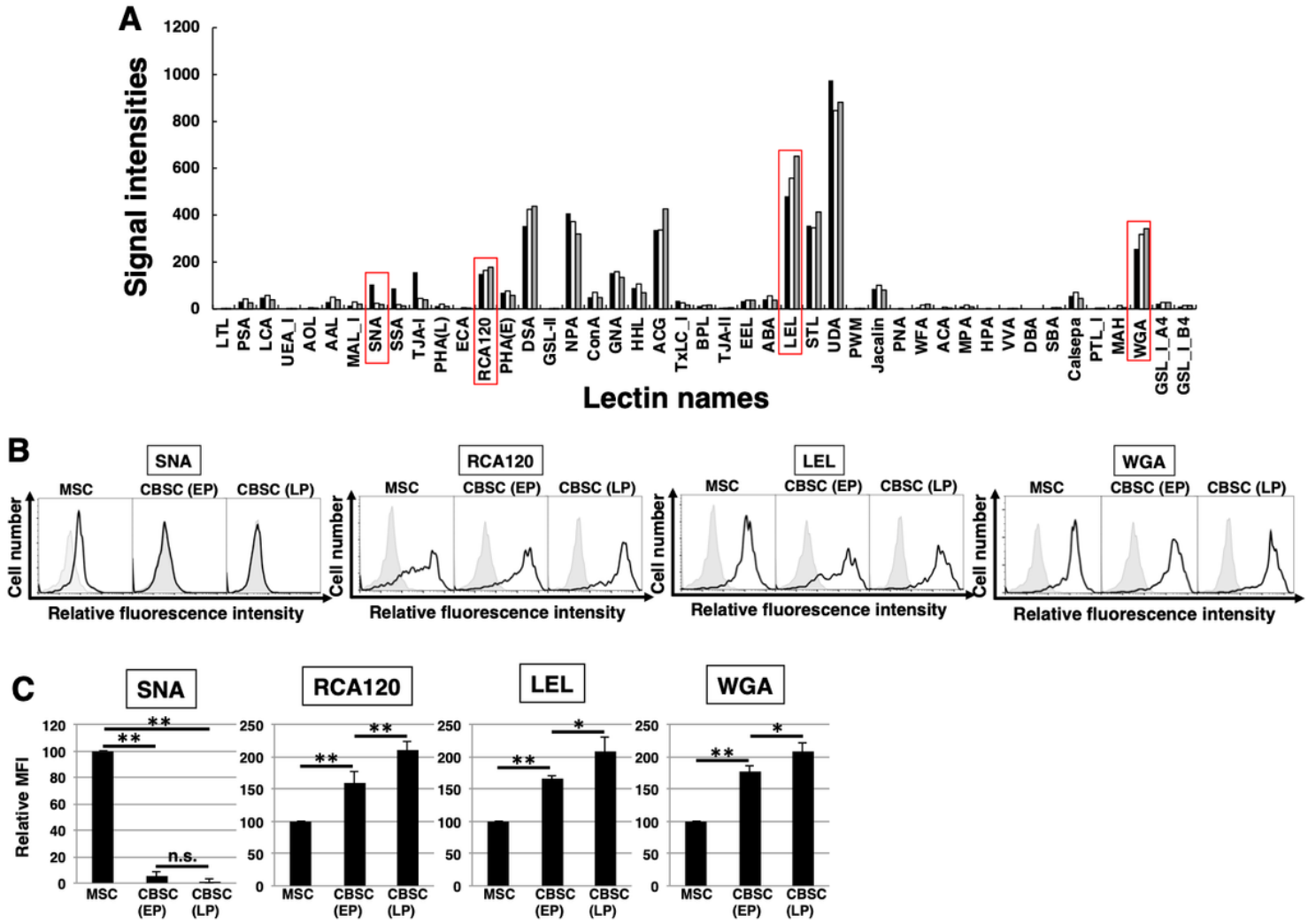

Figure 3

The feature of glycan profiles of mCBSCs and mMSCs. a Glycan profiles of the mMSCs, mouse cortical bone-derived stem cells at early passage (EP-mCBSCs), and those at late passage (LPmCBSCs) by averaged data (n = 6). Bar graph representation of signal intensities of 45 lectins in lectin microarray data. Closed, open, and gray bar showed mMSCs, EP-mCBSCs, and LP-CBSCs, respectively. The lectins enclosed in red line are further experimented in (b) and (c). b FACS analysis of mMSCs, EP-CBSCs, and LP-CBSCs using lectins (SNA, RCA120, LEL, and WGA). Three independent experiments were performed and representative results are shown. Negative control is shown in gray. c MFIs relative to those of mMSCs (value = 100) are shown. Results are presented as means ± SD from three independent experiments. *p < 0.05, **p < 0.01. Abbreviations: mMSC, mouse mesenchymal stem cell; mCBSC, mouse cortical bone derived stem cell; EP, early passage; LP, late passage; SNA, Sambucus nigra; RCA120, Ricinus communis agglutinin I; LEL, Lycopersicon esculentum; WGA, Wheat germ agglutinin; MFIs, Mean fluorescence intensities.

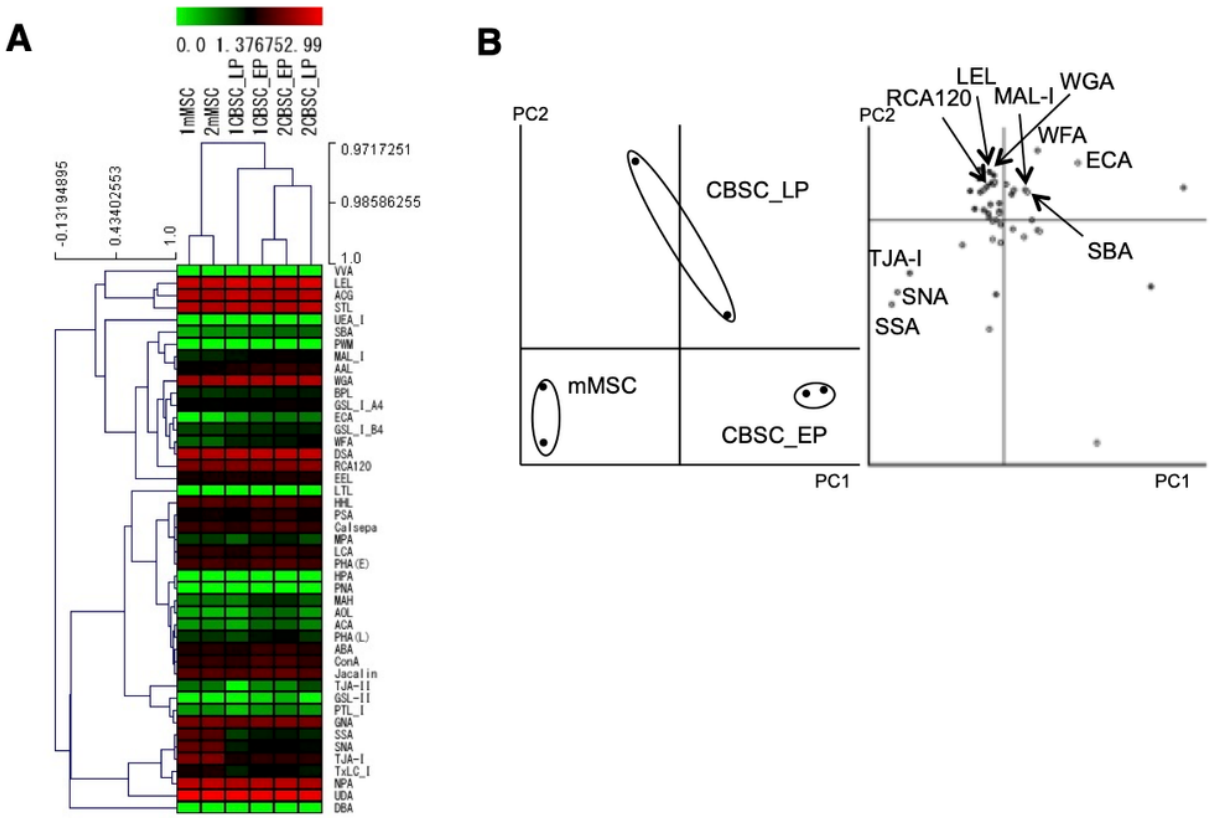

Figure 4

Comparison of glycan profiles between mCBSCs and mMSCs using statistical analysis. a Heat map representation of the (log10-transformed) lectin microarray data. The rows represent the lectins, and the columns represent mMSCs, EP-mCBSCs, and LP-CBSCs with each two cells. The color scale indicates low (green) to high (red) signal intensity. b Biplot for PCA analysis. Left panel: cell replications; right panel: lectin replications shown as a biplot. Abbreviations: mMSC, mouse mesenchymal stem cell; mCBSC, mouse cortical bone derived stem cell; EP, early passage; LP, late passage.

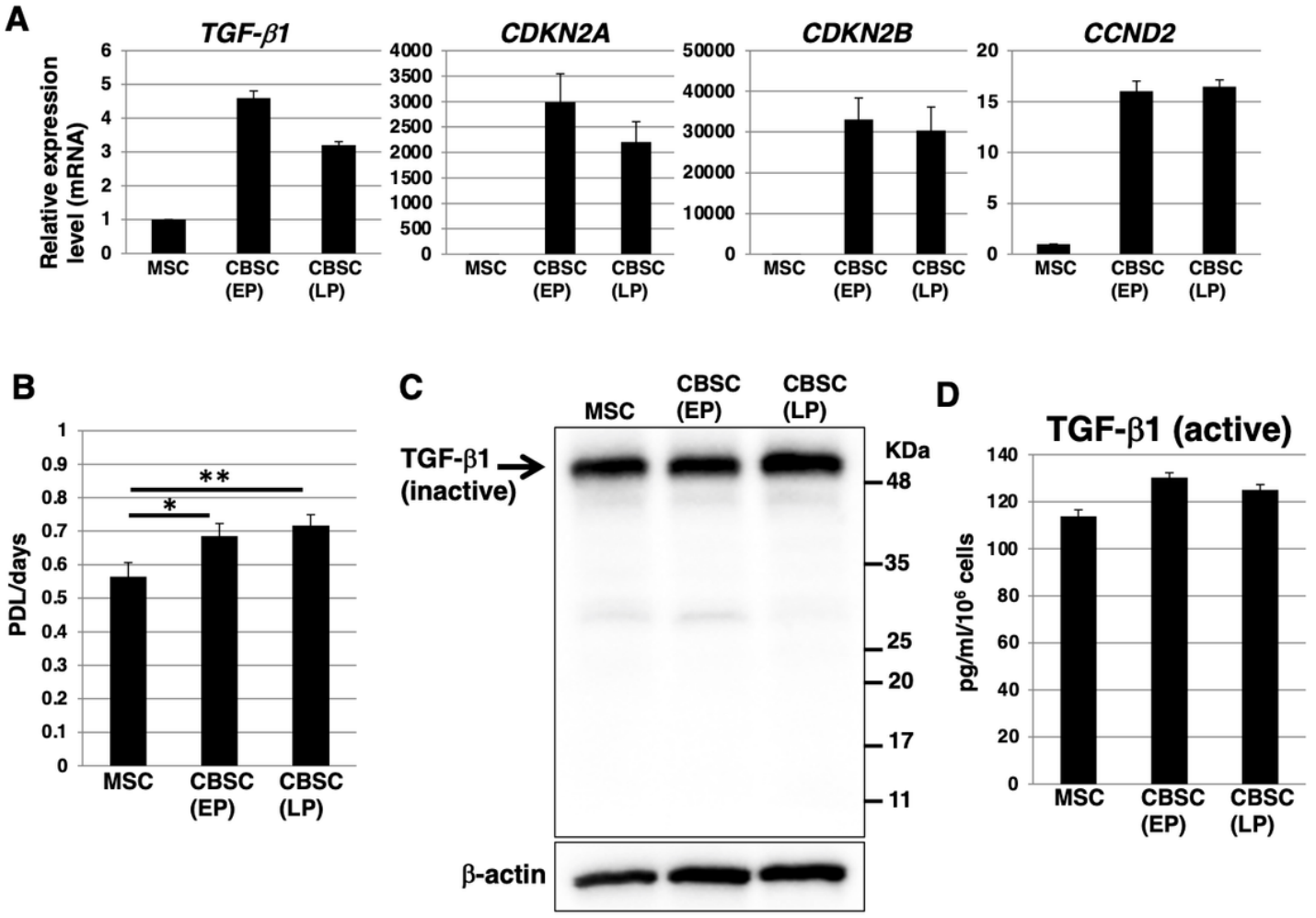

Figure 5

The cell cycle feature and TGF-β1 expression in mCBSCs. a Real-time PCR analysis of TGFβ1, CDKN2A, CDKN2B and CCND2 in mMSCs and mCBSCs (EP and LP). The results are shown after normalization against the values obtained for mMSCs (value = 1). The values shown are the means ± SD from triplicate measurements. b Growth rate in mMSCs and mCBSCs (EP and LP) is shown by PDL/days. Data are expressed as mean ± SD from three independent experiments. *$p < 0.05$; **$p < 0.01$. c Western blot analysis of TGF-β1 and β-actin (loading control) in mMSCs and mCBSCs (EP and LP). d Cell culture supernatant from mMSCs and mCBSCs (EP and LP) was subjected to ELISA detection for TGF-β1 levels. The values shown are the means ± SD from triplicate measurements. Abbreviations: mMSC, mouse mesenchymal stem cell; mCBSC, mouse cortical bone derived stem cell; EP, early passage; LP, late passage; PDL, population doubling level.

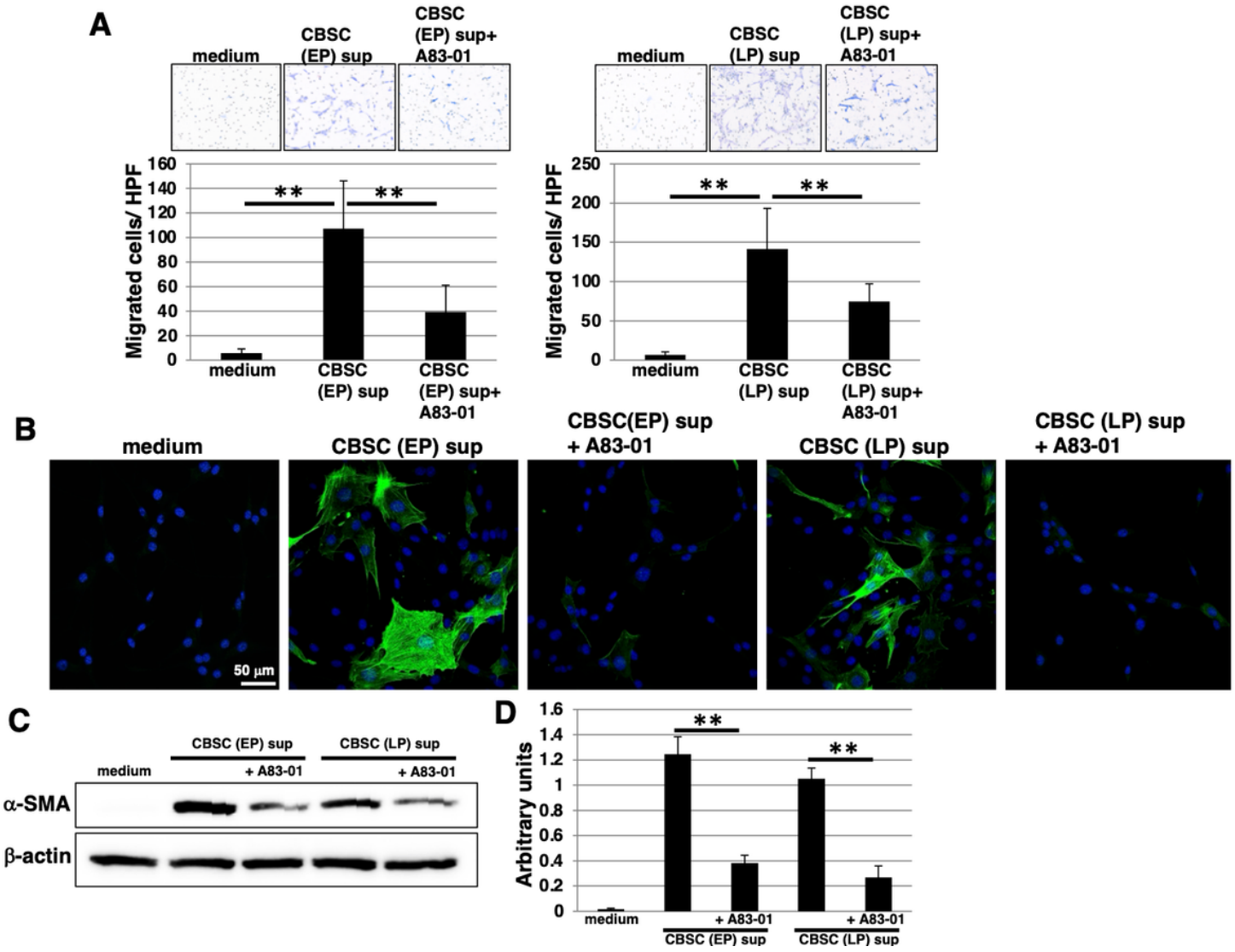

Figure 6

The functional properties of TGF-β1 derived from mCBSCs. a Migration assays were performed in mCBSCs (EP and LP). CBSCs growth media, mCBSCs culture media, or mCBSCs culture media with 1μM A83-01, was used for chemoattractant. Representative results from measurements of 12 fields are shown. **p < 0.01. b Immunocytochemical staining was performed in NIH/3T3 cells cultured with CBSCs growth media, mCBSCs culture media, or mCBSCs culture media with 1μM A83-01. Representative images are shown (α-SMA, green; DAPI, blue). c Western blot analysis of α-SMA and β-actin (loading control) was performed in NIH/3T3 cells cultured with CBSCs growth media, mCBSCs culture media, or mCBSCs culture media with 1μM A83-01. d The histogram shows the mean densitometric analysis ± SD of α-SMA normalized to the loading control (β-actin). The values were obtained from three independent experiments. **p < 0.01. Abbreviations: mMSC, mouse mesenchymal stem cell; mCBSC, mouse cortical bone derived stem cell; EP, early passage; LP, late passage.

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