MP043

IMMORTALIZATION AND IDENTIFICATION OF PORCINE METANEPHRIC MESENCHYMAL CELLS

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INTRODUCTION AND AIMS

The metanephric mesenchymal cells (MMCs) generate the diverse epithelial cell types of the nephron and are useful tools to investigate cell differentiation and organogenesis. However, the limited life span of primary cells obviously restricts their usefulness. Herein, we aim to establish an immortalized MMC cell line which can retain essential features of primary cells and provide steady supply of homogeneous cells for future researches.

METHODS

Primary MMCs were extracted from the kidneys of 10-week-old fetal pigs. The experimental protocol was carried out in accordance with the approved guidelines of the Institutional Animal Care and Use Committee at the Chinese PLA General Hospital. Bmi1 gene was introduced by lentiviral transduction into MMCs (MMC-Bmi1). Expression of Bmi1 and telomerase reverse transcriptase gene (TERT) were measured by real-time PCR. Bmi1, $p16^{INK4a}$ and p27 were studied by immunoblotting. Senescence-associated β-galactosidase (SA-βGal) was assayed by SA-βGal staining. Limiting dilution was used to pick single cell clones for further research. MMC protein markers, mesenchymal and epithelial markers were detected by immunofluorescence staining.

RESULTS

1. Bmi1 overexpressed in MMC-Bmi1. Compared with primary cells, the expression levels of Bmi1 mRNA (Fig 1A) and protein (Fig 1B) increased dramatically in MMC-Bmi1. In addition, the transcription of TERT that encodes the catalytic protein of telomerase had a dramatic increase as well (Fig 1C).

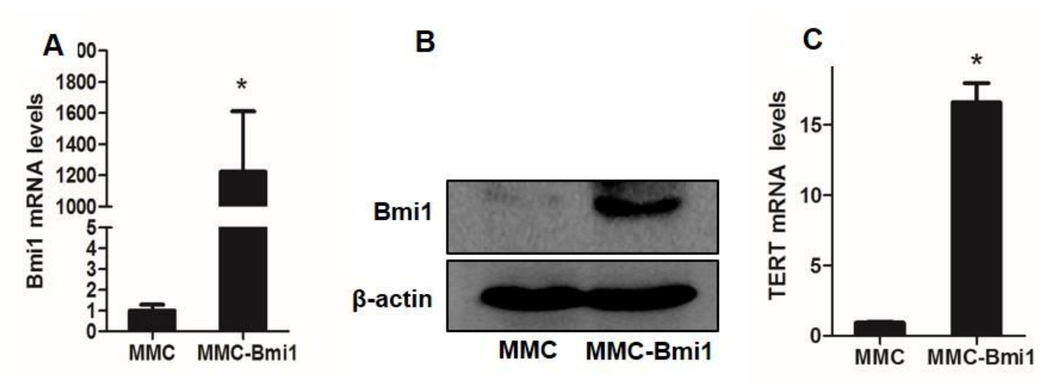

Figure 1. Overexpression of Bmi1 and TERT in MMC-Bmi1. (A-B) The expression of Bmi1 in MMC and MMC-Bmi1; (C) The expression of TERT in MMC and MMC-Bmi1. *, vs MMC, $p<0.05$.

2. The MMC-Bmi1 acquired a slower aging rate. The MMC-Bmi1 could proliferate for more than 50 passages without crisis and the morphology maintained almost the same as the MMCs at early passages (Fig 2A-C). The accumulations of senescence related SA-βGal, $p16^{INK4a}$ and p27 were much less in MMC-Bmi1 cells at passage 30 (P30) than in primary MMCs at P10.

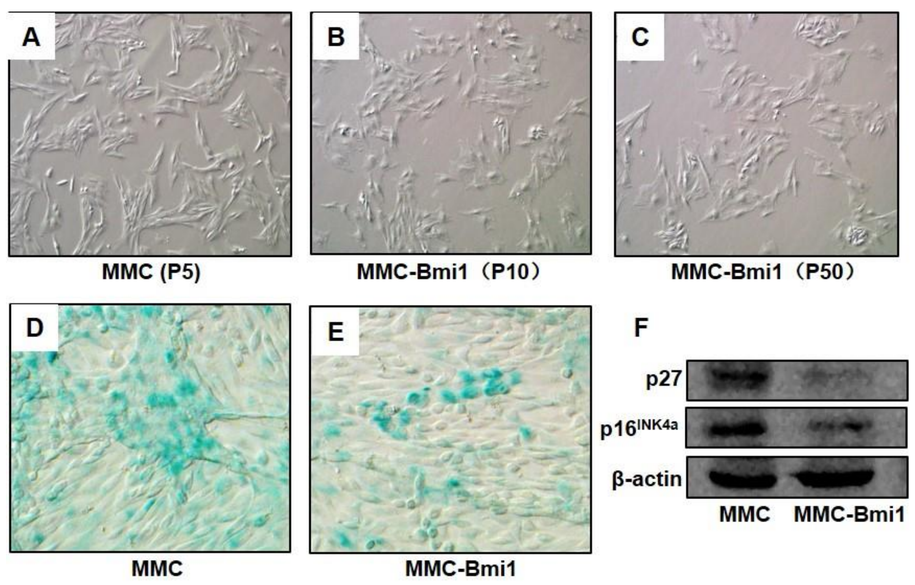

Figure 2. Senescence of MMC and MMC-Bmi1. (A-C) The morphology of MMC and MMC-Bmi1 at different passages (100x). (D-E) SA-βGal staining of MMC and MMC-Bmi1 cells (200x). (F) Expression of $p16^{ink4a}$ and p27 in MMC and MMC-Bmi1 cells.

3.The MMC-Bmi1 retained the phenotype of primary cells. MMC-Bmi1 cell colonies were picked by limiting dilution (Fig 3A-C). Cells that in spindle-shaped morphology were selected for further research (Fig 3D). The results showed that they were positive for MMC markers Six2, Pax2, Gdnf (Fig 3E-G) and mesenchymal marker Vimentin (Fig 3H), but negative for epithelial marker E-cadherin (Fig 3I).

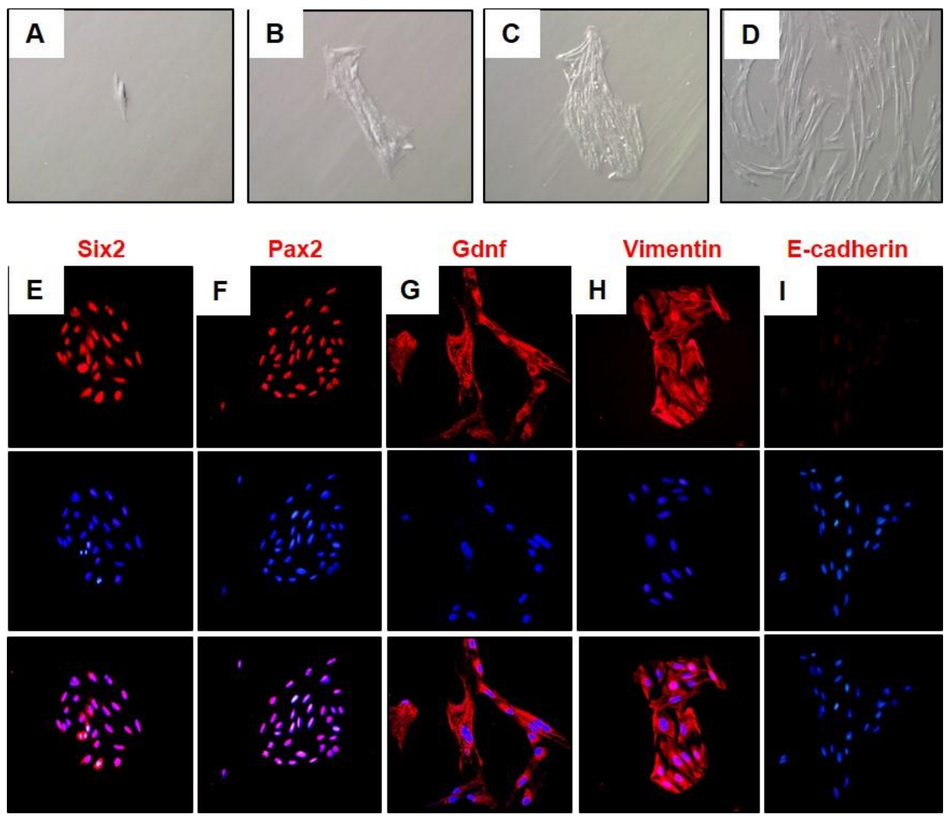

Figure 3. Phenotype of MMC-Bmi1. (A-C) Cell colony derived from a single cell (200x, 100x). (D) The morphology of a selected colony (200x). (E-I) Immunofluorescence staining for Six2, Pax2, Gdnf, Vimentin and E-cadherin (400x）.

4. The MMC-Bmi1 retained the epithelial differentiation ability of primary cells. Under the induction of several cytokines, the spindled-shaped MMC-Bmi1 (Fig 4A-D) changed into cobblestone-like epithelial cells and began to express E-cadherin (Fig 4E-H).

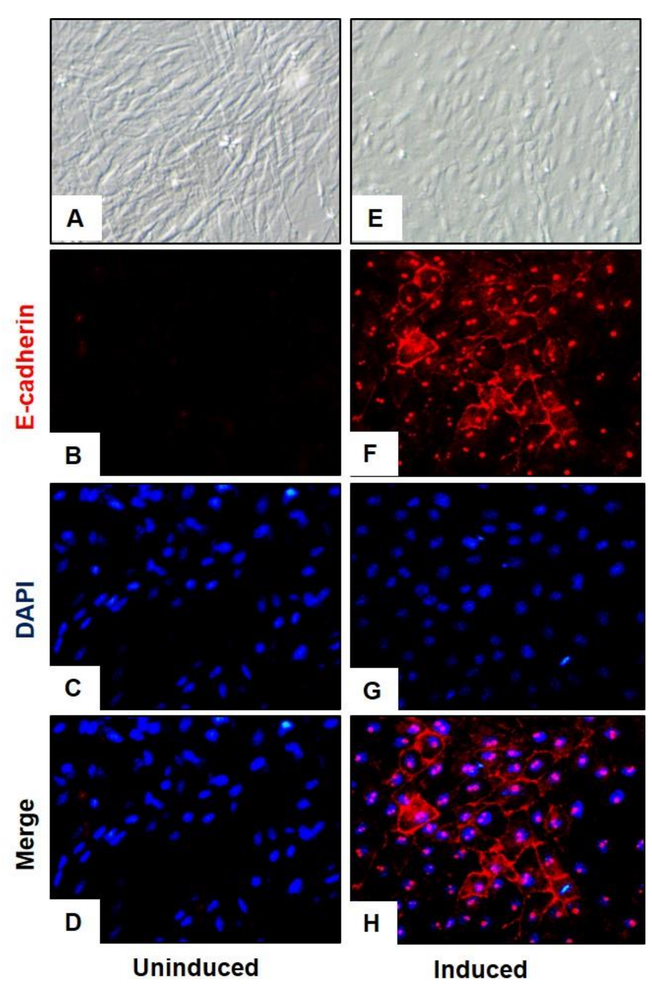

Figure 4. Epithelial conversion of MMC-Bmi1. (A) The spindle shaped MMC-Bmi1 in uninduced condition. (E) MMC-Bmi1 converted into cobblestone-like cells under induced condition. The expression of E-cadherin in uninduced (B-D) and induced (F-H) conditions.(200x)

CONCLUSIONS

The overexpression of Bmi1 was able to extend the life span of porcine MMCs without significantly perturbing their phenotype and biological functions. MMC-Bmi1 cell line may provide a useful tool for the investigations of the directed differentiation and its molecular mechanisms.

DOI: 10.3252/pso.eu.54ERA.2017