

JB Commentary

Roles of Dppa2 in the regulation of the present status and future of pluripotent stem cells

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Tetsuro Watabe*

Tetsuro Watabe, Department of Molecular Pathology; Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

*Tetsuro Watabe, Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel: +81-3-5841-3356; Fax: +81-3-5841-3354, email: t-watabe@umin.ac.jp

Maintenance of undifferentiated states of pluripotent embryonic stem cells is regulated by a complex network of transcription factors and signaling pathways. Recent reports revealed that developmental pluripotency-associated 2 (Dppa2), which regulates chromatin structures, plays important roles in the maintenance of pluripotency and proliferation of embryonic stem cells. Interestingly, developmental pluripotency-associated 2 is involved not only in the normal development of lung but also in the pathogenesis of non-small cell lung cancers. These results suggest that an epigenetic regulator of pluripotent stem cells plays important roles in normal development and tumorigenesis.

Keywords: Dppa2/ES cells/pluripotency/self-renewal/lung/non-small cell lung cancer.

Abbreviations: ES, embryonic stem; Dppa, developmental pluripotency-associated; ECAT, ES cell-associated transcripts; shRNA, short hairpin RNA; NSCLC, non-small cell lung cancers.

Mouse embryonic stem (ES) cells are derived from inner cell mass of blastocysts and pre-implantation embryos, and they are characterized by their ability to propagate indefinitely in culture and to differentiate into various types of cells in culture and in embryos (1). Because of the enormous potential of ES cells for regenerative medicine, understanding the molecular mechanisms underlying their properties of self-renewal and pluripotency is important.

These unique characteristics of ES cells are regulated by a complex regulatory network, with interplay between extrinsic and intrinsic factors. To transmit the states of pluripotency and self-renewal to the daughter cells, the expression levels of the genes that are involved in these mechanisms need to be maintained. Recent extensive molecular studies have revealed that a transcription factor network that is stabilized by

positive and negative regulation between its components plays important roles in this maintenance. These components include pluripotent transcription factors, such as Oct3/4, Sox2 and Nanog, that cooperatively regulate transcription of various effectors such as Utl1 and Sall4. Of note, Oct3/4, Sox2 and Nanog form a regulatory feedback circuit that maintains pluripotency of ES cells. However, apart from these transcription factors, few factors have been identified to be involved in the establishment and maintenance of the pluripotency and self-renewal of ES cells.

To identify such factors, several groups have carried out *in silico* screening for genes whose expression pattern is restricted to pluripotent cells (2–4). Analysis of the mouse expressed sequence tag database by comparing gene representation in pluripotent cells with representation in somatic tissues has generated lists of genes named developmental pluripotency-associated (Dppa) (2) and ES cell-associated transcripts (ECAT) (3), both of which include Dppa2/ECAT15-2 and Dppa4/ECAT15-1 (hereafter referred as Dppa2 and Dppa4, respectively).

Dppa2 and Dppa4 contain a common putative DNA-binding domain, the SAP (SAF-A/B, Acinax and PIAS) motif (Fig. 1A), which has DNA/RNA-binding ability and is involved in chromatin modification (5). Genes encoded by Dppa2 and Dppa4 have a similar exon-intron structure, and are tandemly located on the 16th chromosome in the mouse genome (4). In accordance with the result of *in silico* screening, they are ubiquitously expressed in germinal vesicle stage oocytes and during the cleavage stages of mouse embryogenesis (Fig. 1B). Their expression becomes restricted to the inner cell mass of blastocysts, from which ES cells are derived, and is subsequently decreased. Later, in embryogenesis, they are expressed only in the developing germ line, suggesting that their expression is similar to those of pluripotent markers and marks a subset of pluripotent cells (4). Furthermore, Du and colleagues reported that expression of Dppa2 and other pluripotent markers, such as Oct4 and Nanog in mouse ES cells, decreases on differentiation (6).

The restricted expression of Dppa2 and Dppa4 in pluripotent stem cells and their structure containing SAP motif suggest that they are involved in the maintenance of pluripotency and self-renewal of ES cells. To study its roles in the maintenance of undifferentiated states of ES cells, Dppa2 expression was knocked down by short hairpin RNA (shRNA) (6). Decrease in Dppa2 expression induced differentiation of ES cells as indicated by decreased alkaline phosphatase activity and decreased expression of pluripotent markers in concomitant with increased expression of early differentiation markers. Furthermore, reduced expression of Dppa2 resulted in decreased proliferation of ES cells. shRNA-mediated knockdown of Dppa4 also resulted in differentiation of ES cells (7, 8). These results suggest that Dppa2 and Dppa4 play essential roles in the

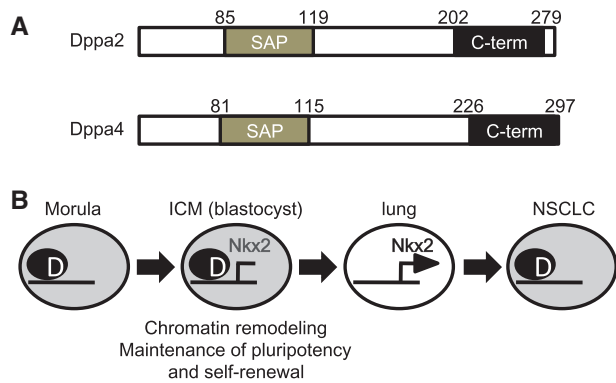


Fig. 1 Dppa2 encodes a SAP domain containing DNA binding protein expressed in pluripotent cells and tumors. (A) Schematic representation of Dppa2 and Dppa4 proteins. The numbers at the N- and C-terminal ends of the SAP and C-terminal (C-term) domains refer to the amino acid residues of mouse Dppa2 and Dppa4. (B) Schematic view of the expression and roles of Dppa2 during development and tumorigenesis. Dppa2 (D) expression is observed throughout in morula and becomes restricted to inner cell mass in blastocyst. Although Dppa2 expression is decreased in somatic cells, Nkx2 gene expression in lung is dependent on the epigenetic modification by Dppa2 in early stages of development. Dppa2 is re-expressed in NSCLC, which may confer the cancer cells with stem cell characteristics.

maintenance of the pluripotency and self-renewal of ES cells *in vitro*.

To study the roles of Dppa2 and Dppa4 *in vivo*, Dppa2/4 double knockout mice were generated (9). Surprisingly, almost all mutant mice died around birth with respiratory defects, suggesting that Dppa2 and Dppa4 play important roles in lung development, and they are dispensable during early development. Although the expression of neither Dppa2 nor Dppa4 was detected in developing lung tissues, Dppa2-null lung exhibited decreased expression of several genes, such as Nkx2-5, Gata4 and Pitx2, that play important roles in lung development. Although the genomic DNA of these genes are in active chromatin statuses in wild-type ES cells, they are inactivated in Dppa2-null ES cells. Because chromatin immunoprecipitation assay revealed that Dppa2 binds to the regulatory region of Nkx2-5 gene in ES cells, epigenetic marks made by Dppa2 in ES cells may play important roles in the development of lung, in which Dppa2 is no longer expressed.

To study the roles of Dppa2 in the maintenance of characteristics of ES cells, Dppa2-null ES cells were generated (9). In accordance with the report by Du and colleagues, the growth of Dppa2-null ES cells was retarded as compared with that of wild-type counterpart. Furthermore, cDNA microarray analysis of Dppa2-null ES cells revealed that the expression of many genes, including those involved in germ cell development, was downregulated (9). Nonetheless, the Dppa2-null ES cells developed normally possibly by compensation mechanisms, by which they can maintain pluripotency and self-renewal, regardless of the altered gene expression. It remains to solve the discrepancy between the knockout mouse and shRNA studies on the roles of Dppa2 in the maintenance of the pluripotency and self-renewal of ES cells.

Recently, a human homolog of Dppa2, DPPA2, was reported to be co-expressed with OCT4 in human ES cells, blastocysts and primordial germ cells (10), suggesting that expression of this gene is evolutionally conserved. Interestingly, in adulthood, DPPA2 transcripts are restricted to normal testis, but become re-expressed in a variety of tumors most notably in 30% of non-small cell lung cancers (NSCLC) (11, 12) (Fig. 1B). These results suggest that DPPA2 plays important roles not only in physiological lung development but also in the pathogenesis of lung cancer.

Cancer cells and pluripotent embryonic cells share many characteristics, such as unlimited proliferation and the ability to self-renew. Because Dppa2 is involved in the proliferation of mouse ES cells (9), its human orthologue may also play a role in the proliferation of lung cancer cells. Taken together with the restricted expression of DPPA2 in tumors, these results suggest that DPPA2 is a promising target for antigen-specific immunotherapy in NSCLC. Furthermore, better understanding of the roles of DPPA2 in the proliferation and maintenance of stem cell activity of cancer cells may lead to the development of new therapeutic approaches for NSCLC.

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Conflict of interest

None declared.

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