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Asymmetric distribution of CRUMBS polarity complex proteins from compacted 8-cell to blastocyst stage during mouse preimplantation development

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ABSTRACT

During mouse preimplantation development, blastomeres are equipotent until polarity establishment at compacted 8-cell stage. The intrinsic nature of polarity is the asymmetric distribution of polarity proteins between inside and outside blastomeres along the direction of apical-basal axis. This study investigated the early developmental temporal and spatial expression of the main CRUMBS polarity complex proteins in the mouse preimplantation embryo. We observed that *Crb3*, *Pals1*, *Patj* and *Mpdz* are transcribed in the mouse preimplantation embryo. However, the asymmetric distribution of these polarity proteins is not established until the compacted 8-cell stage. From compaction and thereafter, CRB3 and PALS1 are progressively enriched in the apical membrane, while PATJ and MPDZ are discretely localized at both tight junctions and the apical membrane adjacent to tight junctions. These temporal and spatial distribution patterns suggest that CRUMBS polarity complex might be involved in the cell polarity establishment in the early mouse embryo and reinforce the viewpoint that developmentally spatial asymmetries are first set up at the compaction stage. The present study provides a foundation for further investigation on the functions of CRUMBS polarity complex in trophectoderm specification and blastocyst morphogenesis.

1. Introduction

Cell polarity is one of the essential properties of mammalian embryos, and it influences the differentiation and cell fate decision of embryos (Saiz and Plusa, 2013; Takaoka and Hamada, 2012; Yamanaka et al., 2006). In the mouse, cell polarity occurs from the compacted 8-cell stage and thereafter (Rossant and Tam, 2009; Yamanaka et al., 2006). The intrinsic nature of polarity is the asymmetric distribution of polarity proteins between inside and outside blastomeres along the direction of apico-basal axis (Yamanaka et al., 2006). Inside the cell, polarity proteins usually exist as the polarity protein complex to play their physiological functions.

CRUMBS complex mainly comprises CRB, PALS1, PATJ and MPDZ polarity proteins. And it was localized in the regions of apical membrane and tight junction (TJ) (Assemat et al., 2008; Harder and Margolis, 2008; Wells et al., 2006; Yi et al., 2011). CRB (CRUMBS) are transmembrane proteins and CRB1, 2, and 3 have been identified in mammals, among which CRB3 is mainly expressed in all epithelial tissues (Assemat et al., 2008; Lemmers et al., 2004; Makarova et al., 2003). CRB proteins contain two motifs, a FERM binding domain and a PDZ binding domain consisting of ERLI residues (Assemat et al., 2008; Lemmers et al., 2004). CRB3 has an additional SH3 binding site (Assemat et al., 2008; Lemmers et al., 2004). PALS1 (Protein Associated with Lin Seven 1) is an evolutionarily conserved scaffold protein with multiple protein-protein interaction domains including two L27 domains, a PDZ domain, an SH3 domain, a hook domain and a GUK domain (Assemat et al., 2008; Kamberov et al., 2000; Schluter and Margolis, 2012). PATJ (Pals-associated tight junction protein) and MPDZ (multi-PDZ domain protein, also called MUPP1) are multiple PDZ domain containing proteins localized at TJs (Assemat et al., 2008). PATJ contains a L27 domain at the N-terminal and followed by up to ten PDZ domains (Lemmers et al., 2002). Similarly, MPDZ has a L27 domain in its N-terminal and 13 PDZ domains (Ullmer et al., 1998). The first L27 domain of PALS1 interacts with L27 of PATJ/MPDZ and the

Abbreviations: TJ, tight junction; qPCR, Real-time quantitative PCR; TE, trophectoderm; HCG, human chorionic gonadotropin

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second with Lin7; PATJ, MPDZ and Lin7 are the key components for formation and maintenance of TJs (Assemat et al., 2008). PALS1 interacts directly with the ERLI motif of the cytoplasmic domain (C-terminal) of CRB via its PDZ domain (Makarova et al., 2003; Roh et al., 2002, 2003).

In mammalian epithelial cells, CRB3 contributes to stabilizing apical cell junctions in epithelial cells. Overexpression of CRB3 in MDCK cells delays the formation of TJs and disrupts polarity in cysts when cultured *in vitro* in a supporting matrix (Lemmers et al., 2004). PALS1 is required for establishment and formation of integral TJs and cell polarity (Assemat et al., 2008; Roh et al., 2003; Straight et al., 2004). Knockdown of PALS1 expression in MDCK cells leads to PATJ or CRB3 expression and localization disorders. And this will result in delay and defects in formation of TJs and cell polarity, and the inability to form luminal cysts (Roh et al., 2003; Straight et al., 2004). Moreover, alteration in PATJ or CRB3 expression can also cause errors in PALS1 localization and defects in TJs and cell polarity (Assemat et al., 2008; Roh et al., 2003; Shin et al., 2005). Downregulation of PATJ disrupts the distribution of CRB3 and PALS1 at the apical membrane and TJs (Lemmers et al., 2002; Michel et al., 2005; Shin et al., 2005). Thus, reciprocal interactions among polarity proteins are crucial for stabilizing the structure of polarity protein complex and maintaining cell polarity.

During preimplantation embryo development, polarity causes different intracellular signal cascades among the blastomeres, and ultimately, leads to distinct lineage fate between inside and outside blastomeres (Cockburn et al., 2013; Hirate et al., 2013; Johnson and McConnell, 2004). The physiological functions of protein factors are closely related with their expression patterns and subcellular localization. Therefore, in this study, we investigated the transcription and subcellular localization of the main CRUMBS polarity complex proteins in early mouse embryos. These findings may provide a foundation to study the biological significance of CRUMBS complex in lineage fate decision of preimplantation embryos.

2. Results

2.1. The mRNA expression of Crb1, 2, 3, Pals1, Patj and Mpdz in the early mouse embryo

First, we examined *Crb3*, *Pals1*, *Patj* and *Mpdz* expression by RT-PCR at each typical developmental stage during preimplantation development. We observed that *Pals1* and *Patj* mRNAs were expressed from the zygote through to blastocyst stage. *Mpdz* was transcribed from compacted 8-cell to blastocyst stage. *Crb3* was not detected in zygotes, whereas it was detectable from the 2-cell to blastocyst stage (Fig. 1A). Yin et al. reported *Crb3* mRNA was detected in zygotes (Yin et al., 2014). This difference might result from specificity of the primers since no sequencing verification of *Crb3* primers was done in Yin et al. paper. There are two other homologues of CRUMBS. Different from *Crb3*, *Crb1* is expressed in the cerebrum and retina (den Hollander et al., 2002) and *Crb2* is expressed at high levels in the kidney and retina and at lower levels in the heart, lungs and placenta (Ja et al., 2005). *Crb2* was detected during preimplantation development, yet *Crb1* can only be examined in zygotes and 2-cell embryos. However, their mRNA levels are rather low in preimplantation embryos (Fig.S1).

We next performed Real-time quantitative PCR (qPCR) experiments to investigate the relative transcription levels of *Crb3*, *Pals1*, *Patj* and *Mpdz* mRNAs. From the 2-cell stage, *Crb3* transcription emerged, and it showed a slight increase at 4-cell stage. At 8-cell stage, *Crb3* transcription reached the highest level, and then mildly declined from compacted 8-cell to blastocyst stage (Fig. 1B). *Pals1* transcription kept in a relatively high level although it had a little slight fluctuation at each stage (Fig. 1C). *Patj* mRNA was also present in preimplantation embryos of each stage, and its transcriptional levels were different among these stages. *Patj* had a moderate expression from zygote to 4-cell stage. From the 8-cell stage onwards, its transcription level had a sharp rise and maintained to blastocyst stage (Fig. 1D). *Mpdz* was first detected in compacted 8-cell embryos, yet the expression level was rather lower than its homolog *Patj*. And at morula and blastocyst stage, *Mpdz* expression decreased gradually (Fig. 1E).

2.2. Asymmetric distribution of CRB3, PALS1, PATJ and MPDZ from compacted 8-cell to blastocyst stage

To investigate CRB3, PALS1, PATJ and MPDZ distribution patterns in mouse preimplantation embryos, we examined their stage-specific localization by immunofluorescence staining. Before compaction, weak and homogenous distribution of these polarity proteins was found throughout the blastomeric cytoplasm of the whole embryo; no specific and asymmetric staining was seen (Fig.S2, S3 and S4). In the compacted 8-cell embryo, morula and blastocyst, polarized distribution appeared along the apical-basal axis. The labeling of CRB3 and PALS1 was observed at the apical membrane (Figs. 2 and 3). PATJ and MPDZ were found at TJs of outer blastomere contacts and were also localized in a discrete state at the apical membrane adjacent to TJs (Figs. 4 and 5). Besides, a very weak staining was also observed for these three proteins at the basolateral membrane in the morula and blastocyst (Figs. 2–4 and 5). To further confirm the localization information, we performed co-staining with adherens junction (AJ) marker E-cadherin or TJ marker ZO-1 in blastocysts. E-cadherin marked AJs and was not localized at the apical membrane, so that the co-staining images clearly demonstrated the apical distribution of CRB3 and PALS1 (Fig. 6A and B). In the outer TE cells, PATJ and MPDZ were present at TJs, overlapped with ZO-1. In addition, their staining was also present at the apical membrane adjacent to ZO-1 (Fig. 6C and D).

Here we showed the polarized distribution of CRB3 in preimplantation embryos. Similar to previous reports, CRB3 is localized at the apical membrane and tight junctions (Assemat et al., 2008; Fan et al., 2004). In Yin et al. study, they found that CRB3 was distributed in the cytoplasm of preimplantation embryos rather than in the polarized apical membrane. Such a difference might be due to the antibody specificity. We tested Santa Cruz CRB3 antibody that Yin et al. used and also observed homogeneous staining throughout the embryo. Theoretically, CRB3 should be localized apically in epithelial tissues (Assemat et al., 2008; Fan et al., 2004). And Novus CRB3 antibody in this study produced specific signals at apical membrane. So the antibody Yin et al. used might not be specific enough for immunofluorescence staining.

3. Discussion

Lineage differentiation is dependent on asymmetric localization of specific proteins inside the cells. CRUMBS polarity complex proteins maintain epithelial cell polarity and provide an interacting platform at TJs and apical membrane for polar signal transduction (Assemat et al., 2008; Roh et al., 2003; Shin et al., 2005; Straight et al., 2004; Wells et al., 2006). During preimplantation development, the Polarity Model, one of the classic lineage fate models, proposes that the apical polarity establishment after 8-cell stage is critical for the first cell fate decision of embryos (Cockburn et al., 2013; Hirate et al., 2013; Johnson and McConnell, 2004). The study described here tracks the early developmental temporal and spatial expression of CRB3, PALS1, PATJ and MPDZ polarity proteins in the mouse preimplantation embryo. Although mRNA could be detected, we did not observe asymmetric localization of these polarity proteins before compacted 8-cell stage, reinforcing that compaction is the appropriate time at which developmentally spatial asymmetries are first established (Rossant and Tam, 2009; Yamanaka et al., 2006). Our results support the observations reported earlier by Vinot et al. on the distribution of PAR3/PAR6/aPKC (another apical polarity complex) in the mouse preimplantation embryo (Vinot et al., 2005). PAR complex proteins do not display an asymmetric distribution before compaction, either.