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Loss of Borealin/DasraB leads to defective cell proliferation, p53 accumulation and early embryonic lethality

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ABSTRACT

Borealin/DasraB is a member of the chromosomal passenger protein complex (CPC) required for proper segregation of chromosomes during mitosis. In *Drosophila melanogaster*, inactivation of Borealin/DasraB results in polyploidy, delayed mitosis and abnormal tissue development, indicating its critical role for cell proliferation. However, the in vivo role of mammalian Borealin/DasraB remains unclear. Here, we analyzed the expression of Borealin/DasraB and found that *borealin* is widely expressed in embryonic tissues and later restricted to adult tissues which relies on rapid cell proliferation. To determine the role of *borealin* during mouse development, we generated *borealin*-null mice through targeted disruption. While heterozygous mice developed normally, disruption of both *borealin* alleles resulted in early embryonic lethality by 5.5 dpc (days postcoitus) due to mitotic defects and apoptosis in blastocyst cells that showed microtubule disorganization and no CPC enrichment. At 5.5 dpc, *borealin*-null embryos exhibited excessive apoptosis and elevated expression of p53. However, loss of p53 did not abrogate or delay embryonic lethality, revealing that Borealin/DasraB inactivation triggered impaired mitosis and apoptosis though p53-independent mechanisms. Our data show that Borealin/DasraB is essential for cell proliferation during early embryonic development, and its early embryonic lethality cannot be rescued by the loss of p53.

1. Introduction

Cell cycle progression is tightly regulated through interphase, mitosis and cytokinesis. During mitosis, successful chromosomal segregation requires dynamic changes of the mitotic spindle assembly, binding of microtubules to the kinetochores, chromosomal arrangement at the midzone and pulling of sister chromatids towards the two spindle poles (Adams et al., 2001; Andrews et al., 2003; Carmena and Earnshaw, 2003; Earnshaw and Bernat, 1991). The CPC is required

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for these mitotic events in organisms ranging from yeast to mammals and consists of at least four components (Vader et al., 2006; Vagnarelli and Earnshaw, 2004): Aurora B, a protein kinase; Inner centromeric protein (Incenp), an activation and targeting subunit; Survivin, an inhibitor of apoptosis-like protein; and the most recently discovered, Borealin/Dasra B (Gassmann et al., 2004; Sampath et al., 2004).

Borealin/Dasra B was identified in human cell lines (Gassmann et al., 2004), *Xenopus* extracts (Sampath et al., 2004) and cultured *Drosophila* cells (Hanson et al., 2005) and was found to co-localize with other CPC proteins throughout mitosis. During the early phases of mitosis, the CPC associates with chromosome arms and centromeres where it then accumulates during metaphase. In anaphase, the complex re-localizes completely to the spindle midzone and midbody of the spindle. Its role at the centromeres and central spindle is thought to be to correct attachment errors at the kinetochore. Evidence suggests that the CPC may be targeted to centromeric DNA directly by its Borealin/DasraB subunit (Rodriguez et al., 2006; Klein et al., 2006). The correct localization of Borealin/DasraB in mitotic cells depends on the function of the other CPC components. RNAi-mediated depletion of Borealin/DasraB results in the mislocalization of Aurora B, Incenp and Survivin and causes multiple mitotic defects including failure in chromosome attachment to the spindle, development of multifocal spindles and uneven chromosome segregation. These defects typically result in multinucleate cells, aneuploidy and polyploidy (Gassmann et al., 2004).

Although previous studies have analyzed the subcellular localization and role of the CPC in somatic cells, the distribution of *borealin* in other tissues and its role in embryonic development remain unclear. Cells that lack *borealin* frequently have delayed apoptosis in *Drosophila* (Hanson et al., 2005). In *incenp*- or *survivin*-null mice, it is not known whether cells with multiple mitotic defects are capable of progressing through the cell cycle by escaping apoptosis. To address these questions, we generated *borealin* knockout mice by homologous recombination. We have found that most knockout mice are embryonic lethal due to a p53-independent fashion.

2. Results

2.1. *Mouse borealin is widely expressed during development*

Through an oligonucleotide array screen for highly expressed genes in neural stem/progenitor cells, we isolated *Cdca8* (recently renamed *borealin*). To investigate the developmental expression of *borealin*, *in situ* hybridizations were performed on sections from C57BL/6 embryonic mice. This revealed a wide but variable expression pattern in different

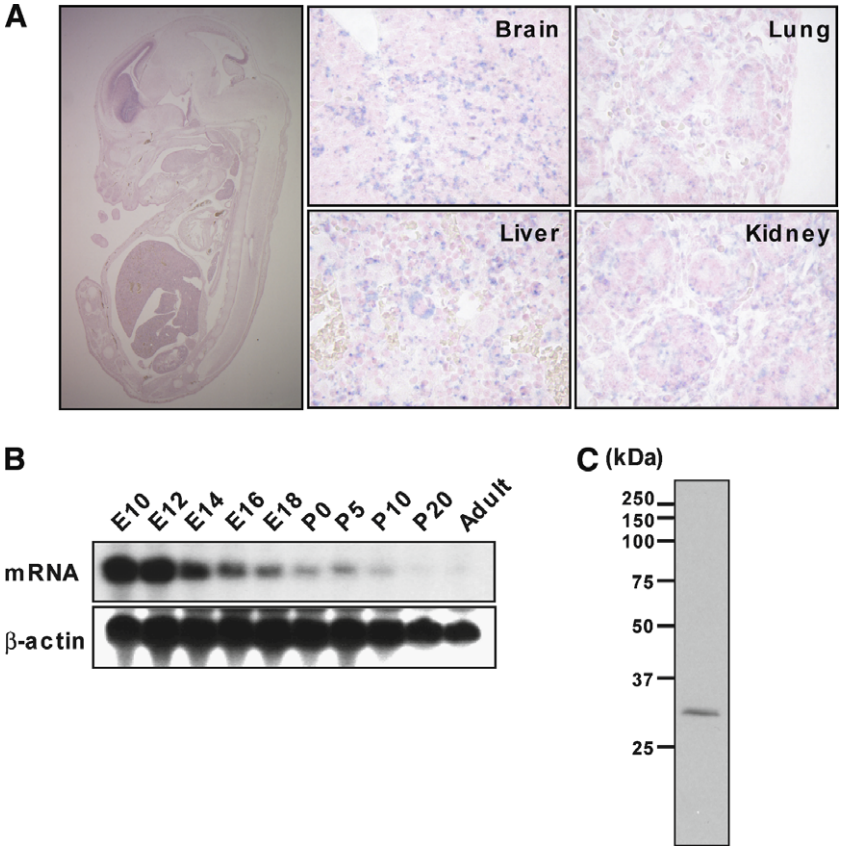

Fig. 1 – *borealin* expression during mouse embryogenesis. *In situ* hybridization analysis of a parasagittal section from a E14.5 embryo with a cRNA probe for *borealin* (A, left panel). Elevated expression levels are observed in the brain, lung, kidney and liver and are shown at higher magnification (A, right panel). *borealin* expression in the mouse brain from E10 to adulthood was detected by Northern blot (B). Borealin/DasraB protein was detected by Western blot using an affinity purified polyclonal anti-Borealin/DasraB antibody (C).