Microtubules and microcephaly: The role of EB2 in brain development

Alexander W. Phillips[1], Cedric Thues[2], Hilde Van Esch[2], David A. Keays[1]
[1] Institute of Molecular Pathology • Vienna Biocenter • Austria
[2] Laboratory for the Genetics of Cognition • KU Leuven • Belgium

IMP Research Institute of Molecular Pathology

Background: The formation of the human brain requires neural progenitors to maintain an equilibrium between proliferative and differentiative divisions. Once differentiation has begun, newly born neurons need to migrate to their proper positions in the developing cortex. The cytoskeleton plays a critical role in mediating these processes. Mutations in genes encoding cytoskeletal proteins have been associated with a wide-range of neurodevelopmental disorders including microcephaly. Recently, it was shown that mutations in microtubule end binding protein 2 (EB2) cause Michelin tire baby syndrome, a congenital disorder characterized by global developmental defects including microcephaly (Isrie et al. 2015). However, the function of EB2 remains poorly characterized. To this end, we will combine transgenic mouse models with stem cell-derived human neural cultures to explore the function of EB2 during brain development.

Aims:

- Assess the expression pattern of EB2 in the developing brain
- Characterise an Eb2 mutant mouse
- Generate EB2 mutant human cerebral organoids to assess the impact of EB2 mutations on human brain development

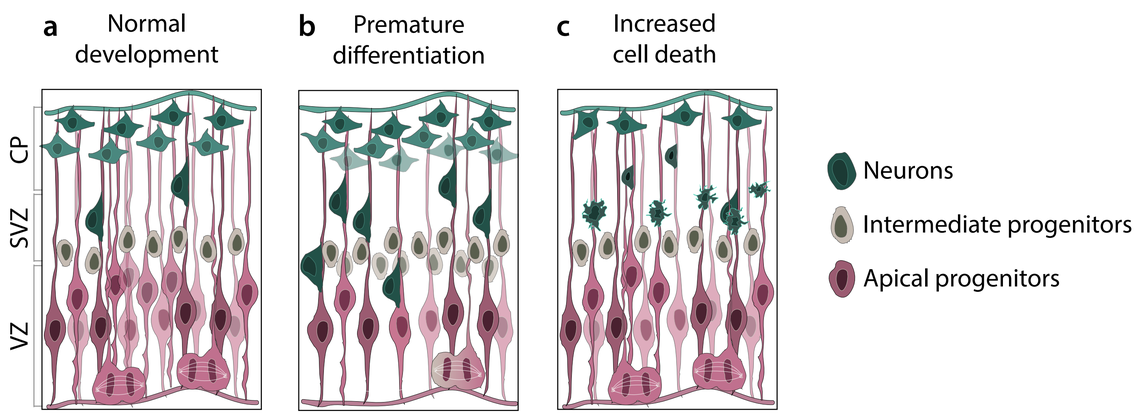

Figure 1. Cellular mechanisms of microcephaly
a. During normal brain development, apical progenitors (red) first divide symmetrically to expand the progenitor pool before differentiating to produce intermediate progenitors (beige) and neurons (green). VZ - Ventricular zone, SVZ - subventricular zone, CP - cortical plate. **b.** Mutations in microcephaly-associated genes such as ASPM and CDK5RAP2 perturb proper mitotic spindle formation leading to premature differentiation and depletion of the progenitor pool (Fish et al., 2006; Lancaster and Knoblich, 2012). Alternatively, mutations in genes important for cell survival and differentiation can cause increased apoptosis in neurons (Ishii et al, 2001; Gstrein et al, 2017).

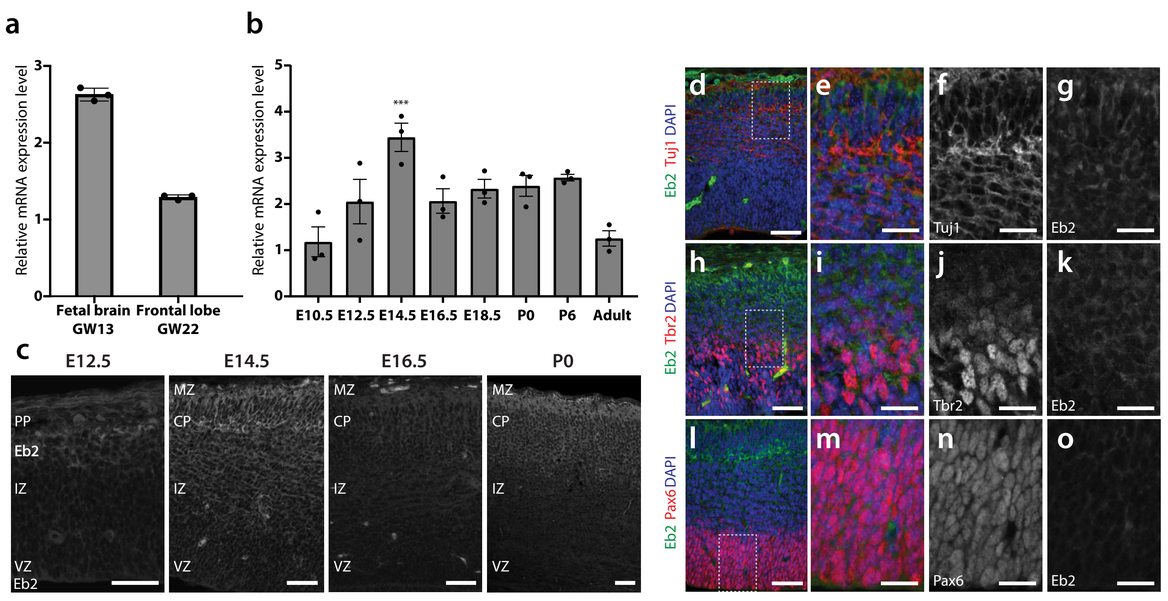

Figure 2. Eb2 expression during brain development
a. qPCR analysis reveals that *EB2* mRNA is expressed in the fetal human brain at gestation week (GW) 13 and 22. **b.** qPCR analysis performed on mouse brain cDNA libraries from E10.5 to adult shows that *Eb2* expression peaks at E14.5 (n = 3 animals per time point). **c.** Immunohistochemistry employing a validated Eb2 antibody shows staining in the ventricular zone (VZ), intermediate zone (IZ) and cortical plate (CP) from E12.5 to P0 in mice (PP - preplate; MZ - Marginal zone). Scale bars 100µm. Immunohistochemistry employing the neuronal marker Tuj1 (**d-g**), the intermediate progenitor marker Tbr2 (**h-k**) and the progenitor marker Pax6 (**l-o**) on E14.5 murine sections reveals that Eb2 is expressed in both progenitors and post-mitotic neurons at this timepoint. Dashed boxes in **d**, **h** and **l** are expanded in **e-g**; **i-k** and **m-o** respectively. Scale bars (**d**,**h** and **l**) 50µm, **e-g**, **i-k**, **m-o** 10µm. Error bars show Mean±SD *** p<0.001 one-way ANOVA with a Bonferroni post test for multiple comparisons.

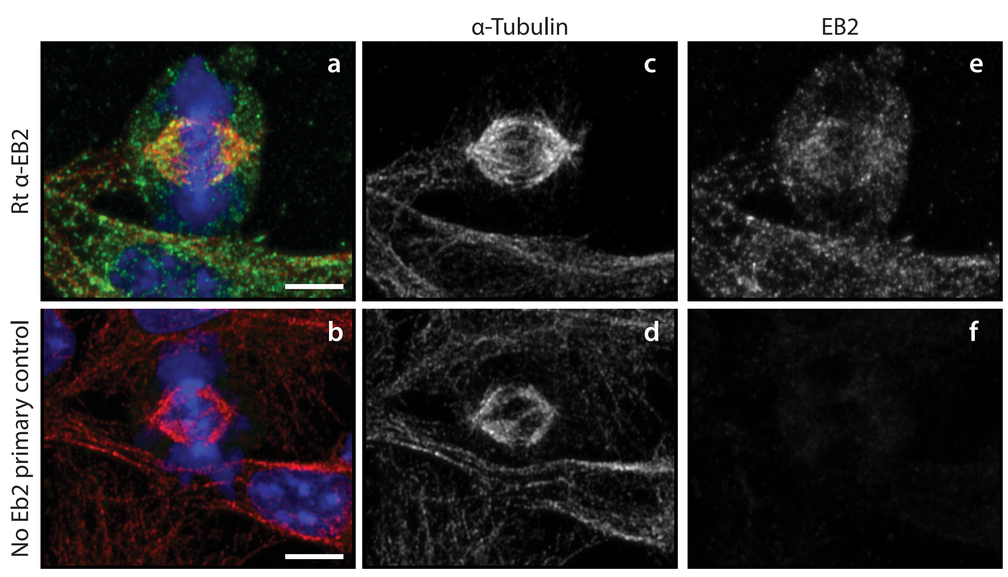

Figure 3. Eb2 localises to the mitotic spindle in mouse neural progenitors
a. Immunohistochemistry employing Eb2 and α-tubulin antibodies shows that Eb2 localisation to the mitotic spindle in mouse neural progenitors. **b.** Negative control staining shows no signal in the absence of Eb2 primary antibody. **c-d** α-tubulin signal from **a.** and **b.** respectively. **e-f** signal from **a.** and **b.**. Scale bars 5µm.

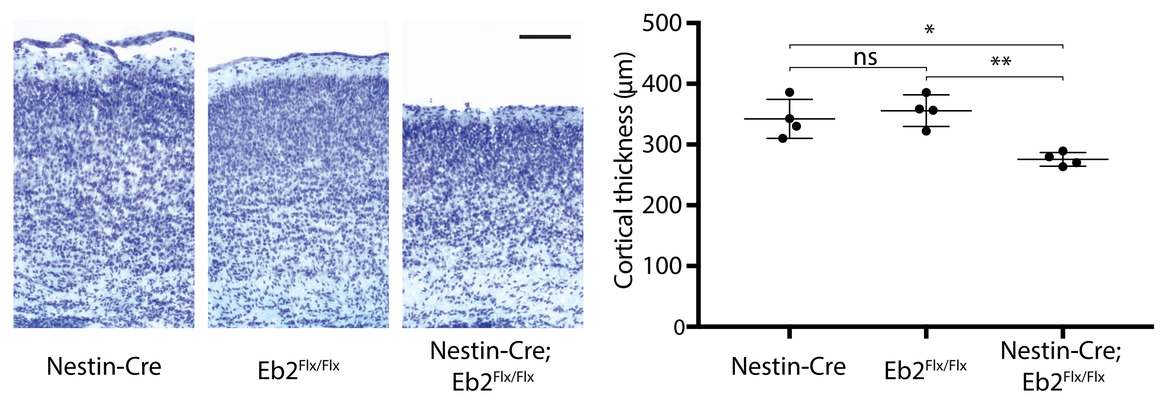

Figure 4. Eb2 mutant mice have a reduction in cortical thickness
a. Nissl-stained coronal brain sections from EB2 littermate control and mutant mice collected at postnatal day 0 of the indicated genotypes. Scale bar 100µm. **b.** Quantification of cortical thickness in matched sections from littermate animals. Error bars show Mean±SD. * p<0.05, ** p<0.01, one-way ANOVA with a Bonferroni post test for multiple comparisons. n = 4 animals for all genotypes.

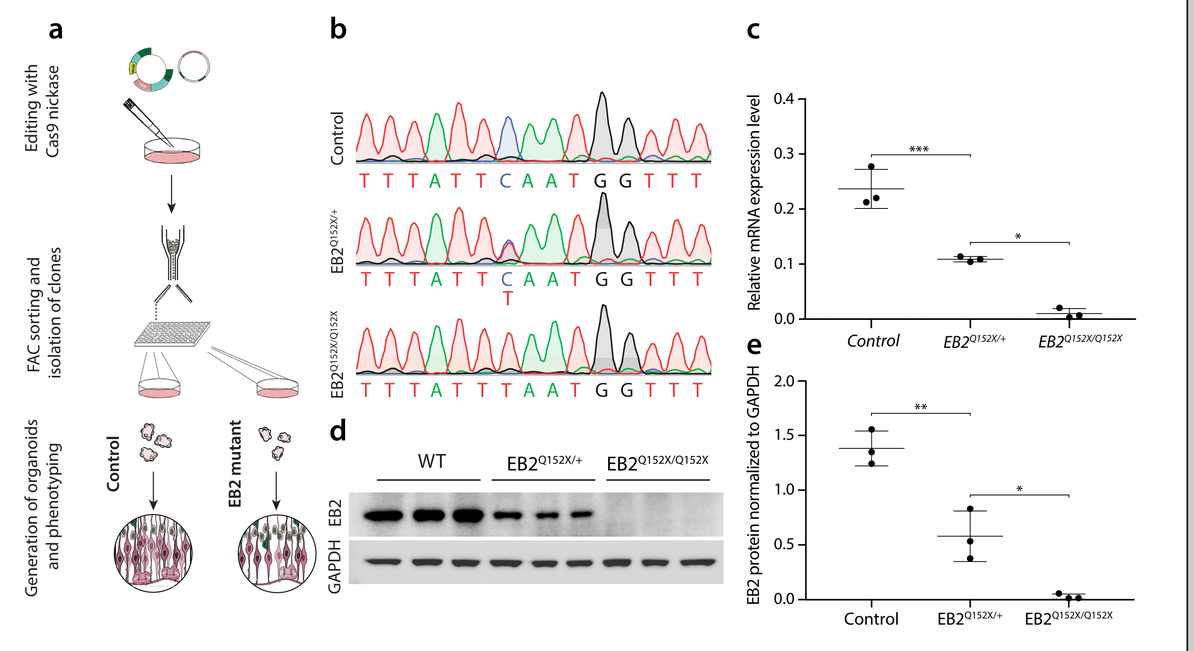

Figure 5. Generation and characterisation of EB2 mutant human stem cells
a. Schematic showing the workflow for generating of EB2 mutant cerebral organoids. Using Cas9 nickase, a patient mutation (Q152X) was knocked into control induced pluripotent stem (iPS) cells alongside excisable EGFP and hygromycin selection cassettes. Cells were sorted for GFP-expression using fluorescence-activated cell (FAC) sorting before plating and antibiotic selection. Control and mutant cells were isolated and verified before organoid generation. **b.** Sanger sequencing traces from control, heterozygous and homozygous EB2 Q152X mutant cells. **c.** qPCR analysis of *EB2* mRNA shows a reduction in the levels of Q152X mutant transcripts compared to control. **d.** and **e.** Western blot analysis of EB2 from iPS cell lysates reveals a significant reduction in protein levels in the mutant stem cell lines. Error bars show Mean±SD. * p<0.05, ** p<0.01, *** p<0.001 one-way ANOVA with a Bonferroni post test for multiple comparisons. n = 3 independent biological replicates for each cell line.

Summary:

- EB2 is expressed in the developing human and mouse brain.
- In embryonic mice, Eb2 is expressed in both neurons and neural progenitors.
- Eb2 localises to the mitotic spindle in neural progenitors.
- Mutations in Eb2 result in a reduction of cortical thickness in mutant mice.
- We have generated EB2 mutant human stem cells for culturing human cerebral organoids.

Outlook:

- Assess whether Eb2 mutations cause premature differentiation or increased cell death in the embryonic mouse brain.
- Generate and characterise EB2 mutant human cerebral organoids.
- Identify EB2 interaction partners.

References

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