ARHGAP11B

Subjects: Cell Biology
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ARHGAP11B is a human-specific gene that likely played a crucial role in human neocortex evolution by inducing hallmarks of cortical expansion. In contrast to its ancestral paralog, ARHGAP11A, ARHGAP11B does not act as a Rho GTPase Activating Protein in the nucleus but is localized in mitochondria and increases glutaminolysis. This increase is a prerequisite for increased basal progenitor proliferation – one essential basis for cortical expansion.

Keywords: microcephaly ; neocortex development ; ARHGAP11B

1. Evolution of ARHGAP11B

ARHGAP11B arose by a partial duplication of *ARHGAP11A* around 5 mya [1][2][3][4]. *ARHGAP11A* is a ubiquitous gene that is composed of 12 exons and encodes a RhoGAP (Rho GTPase Activating Protein) domain-containing protein [5][6]. The partial duplication of *ARHGAP11A* resulting in *ARHGAP11B* encompassed only the first 8 exons of *ARHGAP11A* [7]. This truncated copy of *ARHGAP11A* (referred to as ancestral *ARHGAP11B*) most likely did not contribute to cortical expansion. Indeed, a functional analysis of ancestral *ARHGAP11B* showed that it lacked the ability to promote BP proliferation [7]. Although the *ARHGAP11B* gene is only a truncated copy of *ARHGAP11A*, it contains the full nucleotide sequence of the RhoGAP domain. However, at the protein level, ARHGAP11B features only a truncated RhoGAP domain, lacking the last (C-terminal) 26 amino acids of this domain [7]. In line with this finding, functional analyses showed that ARHGAP11B lacks RhoGAP activity in vivo [8][7]. The truncation of the RhoGAP domain, despite the presence of the full nucleotide sequence for this domain in the *ARHGAP11B* gene, is due to a single C → G nucleotide substitution in the *ARHGAP11B* gene, which most likely occurred after the partial gene duplication event. This substitution generated a novel splice donor site in *ARHGAP11B*, leading to the loss of 55 nucleotides upon *ARHGAP11B* mRNA splicing. This loss led to a frameshift that resulted in a novel, human-specific 47 amino acid-long C-terminal sequence of ARHGAP11B [7]. In contrast to the ancestral *ARHGAP11B* gene that lacks the C → G nucleotide substitution and that would encode an ARHGAP11B protein containing a complete RhoGAP domain and exhibiting RhoGAP activity in vivo, the *ARHGAP11B* gene carrying this substitution, and the truncated RhoGAP domain-containing protein generated from it that can promote BP proliferation (see below), have often been referred to as modern *ARHGAP11B*/ARHGAP11B [7]. For the sake of simplicity, the latter will from now on be referred to merely as "*ARHGAP11B*/ARHGAP11B".

2. Expression of ARHGAP11B during Corticogenesis

During fetal human neocortex development, *ARHGAP11B* is expressed in the VZ and SVZ (including iSVZ and oSVZ), but not in the cortical plate (CP), as shown by in situ hybridization of 13 weeks post-conception (wpc) fetal human neocortex sections [9] and by RNA-seq of micro-dissected germinal zones and CPs of fetal human neocortex ranging from 13 to 16 wpc [10]. Furthermore, RNA-seq data of FACS-isolated cell populations from fetal human neocortex enriched in either apical radial glia (aRG), bRG, or neurons (the latter also containing bRG in G1) showed that *ARHGAP11B* is expressed at almost equal levels in aRG and bRG, but not in neurons [8]. Notably, in contrast to *ARHGAP11A*, *ARHGAP11B* is not expressed in the basal end-feet of radial glia, as shown by in situ hybridization [9][11]. Furthermore, re-analysis of the Florio et al. 2015 RNA-seq data showed that one *ARHGAP11B* splice variant is specifically expressed in bRG [9].

3. Function/Role of ARHGAP11B during Corticogenesis

Functional studies of *ARHGAP11B* in different model systems revealed a potentially important role of this human-specific gene in neocortical development and evolution (summarized in Table 1). Transient overexpression of *ARHGAP11B* in mouse embryos by in utero electroporation led to an increase in BP abundance and proliferation, which was accompanied by an increase in SVZ thickness, and in half of the cases, resulted in folding of the normally unfolded mouse neocortex [8]. Furthermore, transient overexpression of *ARHGAP11B* in ferret embryos by in utero electroporation resulted in an

increase in proliferative bRG, in an extension of the neurogenic period and an increase in upper-layer neurons [12]. Finally, and in contrast to the transient overexpression studies, a recent study with transgenic marmoset fetuses that expressed *ARHGAP11B* under the control of its own human promoter—hence, achieving a physiological-like expression—showed an increased size and the induction of folding of the fetal marmoset neocortex, which at the fetal stage studied, is known to be unfolded [13]. Along with this increase in size and folding, this *ARHGAP11B* expression resulted in an increase in the thickness of the CP and a specific increase in upper-layer neurons. Moreover, the oSVZ—the germinal zone thought to predominantly contribute to human neocortex expansion—was increased in thickness, which reflected an increase in the abundance of BPs, including that of the—for human neocortex expansion, highly relevant—bRG [13]. In summary, gain-of-function experiments with *ARHGAP11B* strongly suggest a key role for this gene in human neocortex expansion.

Table 1. Summary of the main functions of *ARHGAP11B* and of the *NOTCH2NL* genes during neocortex development.

ARHGAP11B	***NOTCH2NLA***	***NOTCH2NLB***
neocortex size ↑	**basal progenitors ↑**	**apical progenitor ↑**
cortical folding ↑	**basal intermediate progenitors ↑**	**cortical progenitor proliferation ↑**
cortical plate thickness ↑		**cortical progenitor differentiation ↓**
upper-layer neurons ↑		**neuronal output ↑**
oSVZ thickness ↑		
basal progenitors ↑		
basal radial glia ↑		

To corroborate the potentially important role of *ARHGAP11B* in fetal human neocortex development, loss-of-function experiments are needed. However, due to the high sequence identity between *ARHGAP11B* and its paralog, *ARHGAP11A* [7], and the expression of these two genes largely in the same cell types [8][9], it is difficult to find guide RNAs for specific and efficient CRISPR/Cas9-mediated disruption of *ARHGAP11B* expression, or small hairpin RNAs for specific *ARHGAP11B* RNAi. To overcome this limitation, it was recently shown that a truncated version of ARHGAP11A that comprises only its N-terminal 220 amino acids (referred to as ARHGAP11A220) can act in a dominant-negative manner on ARHGAP11B's function [14]. Indeed, inhibition of ARHGAP11B's function in fetal human neocortex tissue by ex vivo electroporation using *ARHGAP11A220* was found to result in a decreased abundance of BPs [14], providing the first loss-of-function evidence for an important role of *ARHGAP11B* in fetal human neocortex development.

The same study unraveled the molecular function of ARHGAP11B. In contrast to ARHGAP11A, which is mainly localized in the nucleus, ARHGAP11B is localized in mitochondria due to a functional N-terminal mitochondrial import sequence [14]. Although this mitochondrial import sequence is also present in ARHGAP11A, it appears to be functionally masked in this longer protein. Moreover, ARHGAP11A contains two nuclear localization signals that are not present anymore in the shorter ARHGAP11B protein [14]. In mitochondria, ARHGAP11B interacts with the adenine nucleotide translocase (ANT) and inhibits the opening of the mitochondrial permeability transition pore. As a result, intramitochondrial calcium levels rise, and the metabolic pathway, called glutaminolysis, a hallmark of cancer metabolism [15], is increasingly used [14]. In fact, the ARHGAP11B-induced increase in glutaminolysis was shown to be a prerequisite for the increased BP proliferation upon *ARHGAP11B* expression [14]. Interestingly, the protein encoded by a known microcephaly gene, *MCPH1*, was also shown to be associated with mitochondria and to regulate glutaminolysis [16]. Taken together, these data are consistent with the notion that an increase in glutaminolysis is associated with neocortex expansion, whereas a decrease can be a potential cause of microcephaly [17]. In summary, all the findings discussed in this section point to an important role of *ARHGAP11B* in human neocortex development and most likely in determining its size.

4. Potential Contribution of ARHGAP11B to Microcephaly

ARHGAP11B is located on chromosome 15, near the region that is associated with the 15q13.3 microdeletion/microduplication syndrome. The 15q13.3 microdeletion syndrome is characterized by intellectual and developmental disabilities, mostly delays in speech acquisition and in cognitive function linked to cognitive impairments [18][19][20][21]. Moreover, epilepsy/seizures are present in 28% of affected individuals [21], and the occurrence of schizophrenia was found to be increased in cohorts of 15q13.3 microdeletion patients [21][22]. In some cases, affected patients also display reduced brain volumes and microcephaly [23][24][25]. The 15q13.3 microdeletion is typically characterized by a common 2 Mb deletion on chromosome 15, including the genes *MTMR15*, *TRPM1*, *MTMR10*, *KLF13*, *OTUD7A*, *CHRNA7,* and also *ARHGAP11B* [21]. Some studies have reported homozygous deletion of this chromosomal

region [23][25][26][27][28]. However, a closer examination of the coordinates of the deletions suggested that only one copy of *ARHGAP11B* is included in the deletion, or that *ARHGAP11B* is only very close to the deletion, or that the sequence of *ARHGAP11B's* main isoform is still intact (Table 2).

Table 2. Genomic coordinates (GRCh37/hg19 assembly) of *ARHGAP11B* (left) and of patients reported to have a homozygous 15q13.3 microdeletion (see references indicated).

ARHGAP11B	Le Pichon et al., 2010 [26]	Endris et al., 2010 [23]	Spielmann et al., 2011 [27]	Le Pichon et al., 2013 [28]	Masurel-Paulet et al., 2014 [25]
chr15:30,918,879–30,931,023	chr15:30,931,644–32,914,281	chr15:29,085,644–32,511,004 (paternal) [1] chr15:31,122,986–32,511,004 (maternal) [1]	chr15:30,971,330–32,439,084	chr15:30,931,644–32,914,281	chr15:30,938,215–32,510,863 (three patients)

[1] Patient 2.

Therefore, *ARHGAP11B*'s contribution to 15q13.3 microdeletion/microduplication syndrome as well as to microcephaly, in general, remains open. Due to *ARHGAP11B*'s likely role in human neocortex development and evolution (Table 1), it is conceivable that its mutation or deletion could contribute to microcephaly. Although *ARHGAP11B* has not been the focus of microcephaly studies in the past, it should be noted that, due to its high sequence homology to *ARHGAP11A*, mutations in *ARHGAP11B* in microcephaly patients were perhaps masked by *ARHGAP11A*. As some cases of 15q13.3 microdeletion display microcephaly and *ARHGAP11B* is close to one end of the deletion, it would be important to (re-)analyze 15q13.3 microdeletion cases with microcephaly in detail to see if *ARHGAP11B* is perhaps affected by the deletion.

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