Published in final edited form as:
Autophagy. 2009 February ; 5(2): 259–262.

Insights from DEspR-deficiency and haploinsufficiency

Victoria L.M. Herrera[1], **Julius L. Decano**[1], **Martin Steffen**[2], and **Nelson Ruiz-Opazo**[1,*]

[1] Section Molecular Medicine; Department of Medicine; Boston University School of Medicine; Boston, Massachusetts USA

[2] Department of Pathology; Boston University School of Medicine; Boston, Massachusetts USA

Abstract

We recently showed that DEspR-haploinsufficiency resulted in increased neuronal autophagy and spongiform changes in the adult brain especially the hippocampus, cerebral cortex and basal ganglia, causing cognitive performance deficits. This model demonstrates a causal link between increased autophagy and neurodegenerative changes. This is in contrast with recent observations that decreased autophagy from null mutations of autophagy genes, *Atg5* and *Atg7*, results in early neurodegenerative changes. With the observed autophagy phenotype, we then compared the neural tube phenotype of DEspR-deficient mice with knockout mice of genes established to underlie or regulate autophagy. Intriguingly, the hyperproliferative neuroepithelium observed in DEspR-deficient embryos is also detected in null mutants of *Ambra1*, an autophagy modulator, and two apoptosis genes, *Apaf1* and *Caspase 9*. While all four knockout models exhibited hyperproliferative neuroepithelium, DEspR-deficient mice differed by having greater neural tube cavitation. Additionally, observed DEspR roles in angiogenesis and autophagy recapitulated the association of angiogenesis inhibition and increased autophagy as observed for endostatin and kringle5, thus elucidating an expanding complex network of autophagy, apoptosis and angiogenesis in neuroepithelial development, and an emerging complex spectrum of autophagy effects on neurodegeneration. Nevertheless, DEspR provides a ligand-activated receptor system to modulate autophagy—be it to increase autophagy by inhibition of DEspR-function, or to decrease autophagy by agonist stimulation of DEspR-function.

Keywords

autophagy; neural development; neurodegeneration; spongiform changes; DEspR

With an emerging critical role in neural development,[1,2] it is not surprising that macroautophagy (autophagy hereafter) is implicated in the pathogenesis of age-related neurodegenerative diseases.[3–5] However, cumulative research reveals an emerging complexity evident from the fact that both decreased and increased autophagy are implicated or hypothesized to contribute to age-related neurodegenerative diseases. Decreased autophagy has been implicated in age-related neurodegenerative diseases that are characterized by accumulation of aggregate-prone aberrant protein, such as Alzheimer, Huntington and Parkinson diseases.[3,4] However, these, along with transmissible spongiform encephalopathies such as Creutzfeld-Jacob disease, have all been associated with increased autophagy marked by the accumulation of autophagosomes in affected brains.[5,6]

The recent observation of the association of increased autophagic vacuolization, neuronal loss and spongiform changes in the hippocampus and cerebral cortex of adult mice haploinsufficient

*Correspondence to: Nelson Ruiz-Opazo; Boston University School of Medicine; Section Molecular Medicine; 700 Albany Street, W-609; Boston, Massachusetts 02118 USA; nruizo@bu.edu.

for the dual endothelin-1/vascular endothelial growth factor signal peptide receptor (*DEspR*) gene provides experimental evidence for the association of markedly increased and/or dysregulated autophagy and neurodegenerative spongiform changes in the brain with corresponding cognitive deficits.[7] Intriguingly, decreased numbers of neural progenitor-like subgranular zone cells in the dentate hilar region of the hippocampus were also observed.[7] These observations, which link DEspR haploinsufficiency with autophagic, spongiform neurodegenerative changes, are in contrast with the hyperproliferative, convoluted neuroepithelial phenotype observed in *DEspR* null-deficiency in E12.5-embryonic mouse brain.[8] This difference, therefore, prompted us to compare the neural tube developmental phenotype of established autophagy gene knockouts with the phenotype in DEspR-deficient knockout mice.

An Intriguing Commonality: Hyperproliferative Neural Tube Neuroepithelium

Distinct from other angiogenesis gene knockout mouse phenotypes including the *VEGF*$^{+/-}$ knockout mouse, *DEspR* null mutants also exhibited a hyperproliferative neuroepithelium.[8] We therefore investigated other mice that also exhibited a hyperproliferative neuroepithelium in their respective null mutants. Intriguingly, the gene pathways also exhibiting a hyperproliferative neuroepithelial phenotype are genes involved in autophagy (*Ambra1*) and apoptosis (*Apaf1*, *Caspase 9*).[1,9] As shown in Figure 1, in contrast to the wild-type E12.5 mouse brain, the null mutation of Ambra1, a regulator of autophagy, and Apaf1, an apoptosis regulator, results in extensive overgrowth of the proliferative neuroepithelium in the brain and spinal cord.[1] Notably, however, Ambra1, Apaf1 and also Caspase 9 mutant mouse brains exhibit reduced cavitation of the ventricular system, and mid-hindbrain exencephaly along with the hyperproliferative neuroepithelium,[1,9–11] in contrast to *DEspR*-null mutant brains, which exhibit cavitation despite the convoluted, hyperproliferative neuroepithelium, and which do not exhibit exencephaly (Figs. 1 and 2A–D).[8] High magnification analysis of *DEspR*-null mutant brains reveals hyperproliferation in the ventricular zone, but lack of layering of the developing cortex (Fig. 2C and D) in contrast to wild-type mouse brains, which exhibit distinct layering distinguishing the ventricular, subventricular and intermediate zones (Fig. 2E and F). Analysis of E10.5 *DEspR*$^{-/-}$ embryos by ultrasound micro-imaging detected abnormal telencephalic development and areas of increased grayscale intensity, which most likely correspond to hyperproliferative areas (Fig. 3). We hypothesize that the hyperproliferation in the ventricular zone coupled with a lack of layering suggests a perturbation of asymmetric division and of intermediate progenitor cell proliferation and differentiation.

We note however, that not all null mutations of autophagy genes and apoptosis genes result in a hyperproliferative neuroepithelial phenotype.[4] Rather than a hyperproliferative neuroepithelium, nestin-Cre conditional knockouts of two autophagy genes, *Atg5* and *Atg7*, resulted in early-onset neurodegeneration and progressive deficits in motor function, respectively.[12,13] The association of autophagy and neurodegeneration is also seen in DEspR-haploinsufficient mouse brains, except that instead of decreased autophagy associated with neurodegeneration as seen in *Atg5* and *Atg7* conditional neuronal/glial knockouts, increased autophagy is instead associated with neurodegenerative spongiform changes in the hippocampus and cerebral cortex, with no accompanying inflammatory changes, and minimal if any neuronal ischemia.[7] Altogether, these observations cumulatively corroborate the role of autophagy in neurodevelopment and neurodegeneration, and more significantly, elucidate an emerging complex network with participation of autophagy modulators (DEspR, Ambra1), apoptosis regulators (Apaf1) and mediators (Caspase 9), as well as angiogenesis players (DEspR).

DEspR-Haploinsufficiency Recapitulates the Association of Angiogenesis Inhibition and Autophagy

Adding another level to the emerging complexity of autophagy regulation is the functional association of angiogenesis inhibition and autophagy. Two inhibitors of angiogenesis, endostatin[14] and Kringle 5 of human plasminogen[15] have been described to increase autophagy. Similarly, DEspR, the dual endothelin-1/vascular endothelial growth factor signal peptide receptor, whose null mutation results in marked inhibition of extraembryonic and embryonic angiogenesis,[8] exhibits increased neuronal autophagy in *DEspR*$^{+/-}$ haploinsufficient mice.[7] Because the proliferative neuroepithelial phenotype of *DEspR* null mutants is distinct from *VEGF*$^{+/-}$ knockout mice, we hypothesize that the changes in autophagy most likely pertain to a direct DEspR-mediated modulation of autophagy function rather than through angiogenesis deficits. This hypothesis is supported by the observation that DEspR signaling activates inhibitors of mTOR, the key inhibitor of autophagy, thus resulting in increased autophagy.[7]

Summary

In summary, the causal relationships demonstrated by gene-targeting experiments demonstrate that both a decrease and an increase in autophagy can lead to changes in neuroepithelial proliferation and neurodegeneration via different genes: *Ambra1*, *Apaf1*, *Caspase 9* and *DEspR*. The elucidation of the interactions of these genes and the relationship of angiogenesis inhibition and autophagy, as represented by DEspR, endostatin and kringle5, is important and requires further study. The intriguing association of DEspR-deficiency with hyperproliferative neuroepithelium on the one hand, and DEspR-haploinsufficiency with adult-onset spongiform changes on the other, suggest the hypothesis that DEspR-mediated modulation of autophagy has an impact upon both neuronal development and maintenance.

Precedence for a gene-specific mechanism of aging-associated spongiform changes has been observed in a Drosophila mutant, *Spongecake*, which exhibits region-specific spongiform degeneration in aged flies.[16] As a translational corollary, changes in DEspR expression levels (50% or haploinsufficiency) could lead to nonischemic/noninfectious spongiform changes, thus providing a putative mechanism for microvacuolation and spongiform changes observed with aging-related cognitive impairment. More importantly, modulating DEspR levels and/or function could provide a new pathway to modulate autophagy, as well as autophagy-angiogenesis interactions.

Acknowledgments

This work was supported by NIH grant HL086532 to N. Ruiz-Opazo.

References

1. Cecconi F, Piacentini M, Fimia GM. The involvement of cell death and survival in neural tube defects: a distinct role for apoptosis and autophagy? Cell Death Differ 2008;15:1170–7. [PubMed: 18451869]
2. Cecconi F, Levine B. The role of autophagy in mammalian development: cell makeover-rather than cell death. Cell 2008;15:344–57.
3. McCray BA, Taylor JP. The role of autophagy in age-related neurodegeneration. Neurosignals 2008;16:75–84. [PubMed: 18097162]
4. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27–42. [PubMed: 18191218]
5. Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ. Potential therapeutic applications of autophagy. Nature Reviews Drug Discovery 2007;6:304–12.

6. Williams A, Jahreiss L, Sarkar S, Saiki S, Menzies FM, Ravikumar B, Rubinsztein DC. Aggregate-prone proteins are cleared from the cytosol by autophagy: therapeutic implications. Curr Top Dev Biol 2006;76:89–101. [PubMed: 17118264]
7. Herrera VLM, Decano JL, Bagamasbad P, Kufahl T, Steffen M, Ruiz-Opazo N. Sex-specific hippocampus-dependent cognitive deficits and increased neuronal autophagy in DEspR haploinsufficiency in mice. Physiol Genomics 2008;35:316–29. [PubMed: 18780760]
8. Herrera VLM, Ponce LRB, Bagamasbad P, VanPelt BD, Didishvili T, Ruiz-Opazo N. Embryonic lethality in Dear gene deficient mice: new player in angiogenesis. Physiol Genomics 2005;23:257–68. [PubMed: 16293765]
9. Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, Corazzari M, Fuoco C, Ucar A, Schwartz P, Gruss P, Piacentini M, Choudhury K, Cecconi F. Ambra1 regulates autophagy and development of the nervous system. Nature 2007;447:1121–5. [PubMed: 17589504]
10. Cecconi F, Di Bartolomeo S, Nardacci R, Fuoco C, Corazzari M, Giunta L, Romagnoli A, Stoykova A, Chowdhury K, Fimia GM, Piacentini M. A novel role for autophagy in neurodevelopment. Autophagy 2007;3:506–8. [PubMed: 17622796]
11. Kiuda K, Haydar RF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 1998;94:325–37. [PubMed: 9708735]
12. Hara T, Nakamura K, Matsui M, Yamamoto Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 2006;441:885–9. [PubMed: 16625204]
13. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 2006;441:880–4. [PubMed: 16625205]
14. Chau YP, Lin SY, Chen JH, Tai MH. Endostatin induces autophagic cell death in EAhy926 human endothelial cells. Histol Histopathol 2003;18:715–26. [PubMed: 12792883]
15. Nguyen TM, Subramanian IV, Kelekar A, Ramakrishnan S. Kringle 5 of human plasminogen, an angiogenesis inhibitor, induces both autophagy and apoptotic death in endothelial cells. Blood 2007;109:4793–802. [PubMed: 17272502]
16. Min KT, Benzer S. *Spongecake* and *eggroll*: two hereditary diseases in Drosophila resemble patterns of human brain degeneration. Current Biology 1997;7:885–8. [PubMed: 9382801]

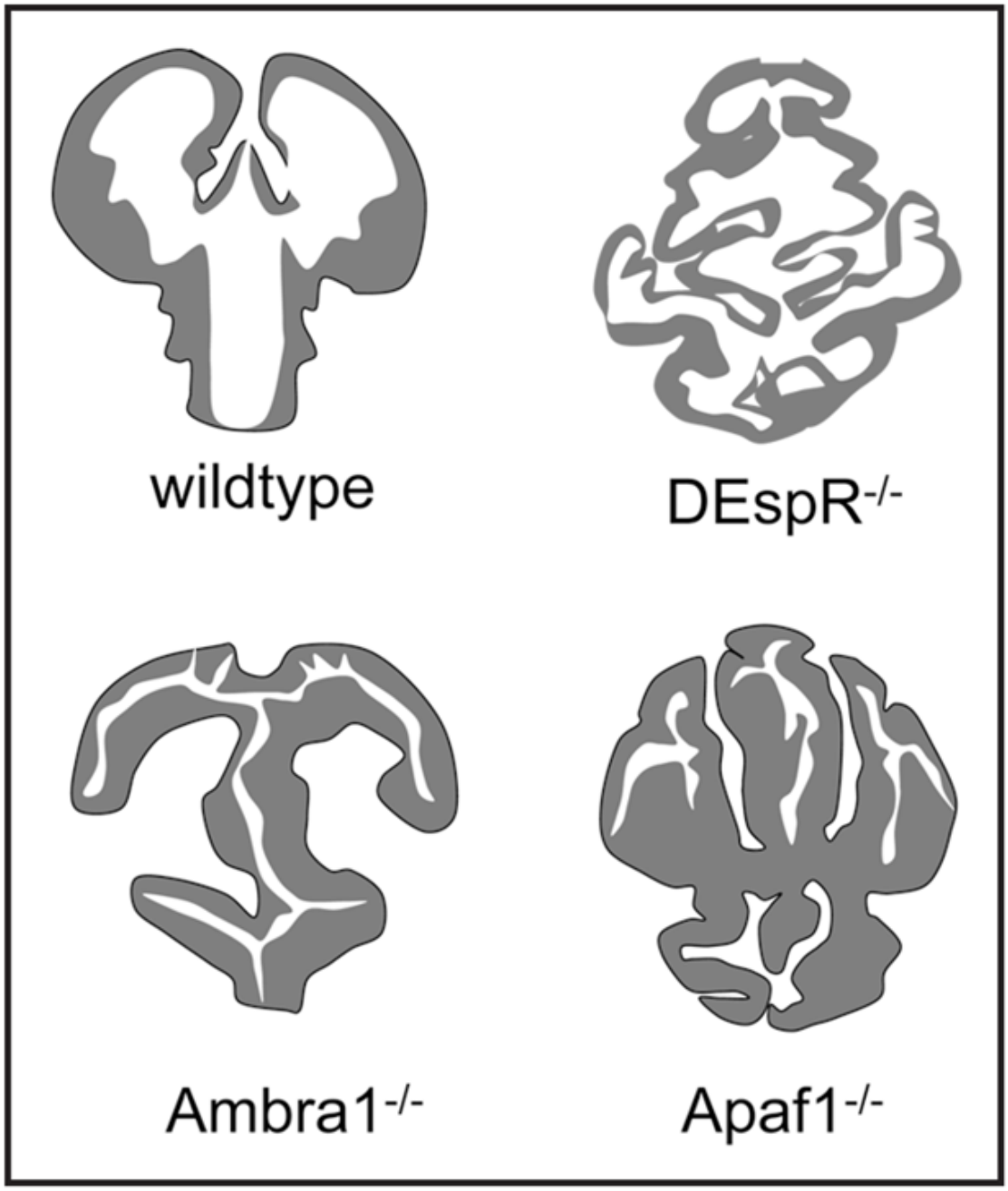

Figure 1.
Comparative analysis of neural tube development in *DEspR*, *Ambra1* and *Apaf1* null mutant mice in contrast to wild-type mouse brains. Schematic representation of coronal views of the E12.5-day embryonic telencephalon and diencephalon of *DEspR*, *Ambra1* and *Apaf1* gene knockout mouse brains, depicts distinct morphologies of null mutant brains exhibiting different degrees of hyperproliferative neuroepithelium (gray) and cavitation (white).

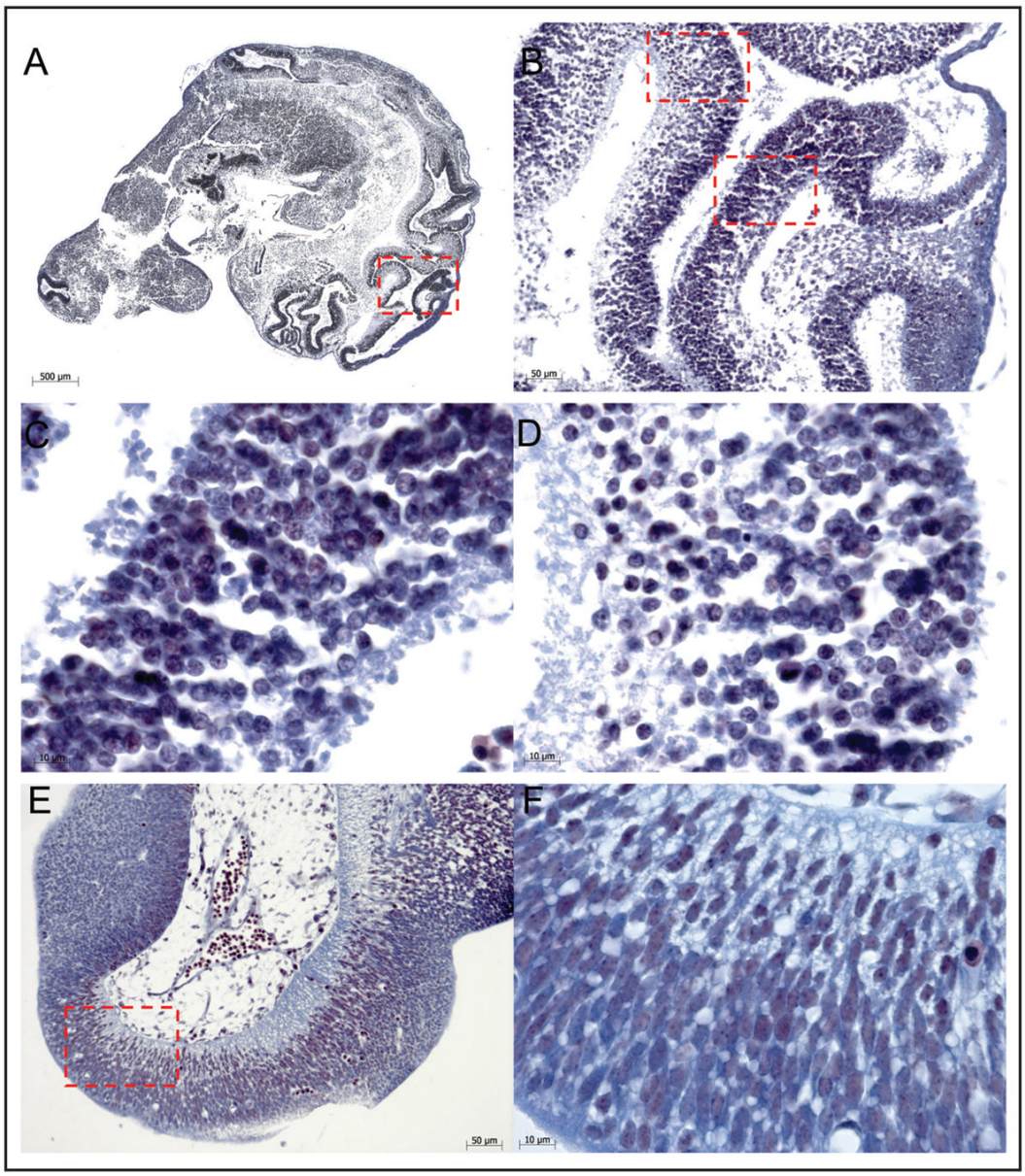

Figure 2.

Histological analysis of E12.5 *DEspR* null mutant embryo compared with littermate wild-type embryo (Masson Trichrome staining). (A) DEspR-deficient mouse embryo exhibiting a convoluted and hyperproliferative neural epithelium affecting the full length of the neural tube. Boxed area magnified in (B). (B) High magnification of the telencephalic region, with two boxed areas for higher magnification analysis in (C and D). (C and D) Hyperproliferation with increased cellular density detected in the abventricular zone, with minimal to no layering. (E) Wild-type mouse neuroepithelium exhibiting neuroepithelial layering and differentiation, and prominent microvessels. (F) High magnification of boxed area in (E) showing prominent layering of the developing brain. Scale bars: (A), 500 microns; (B and E), 50 microns, (C, D and F): 10 microns.

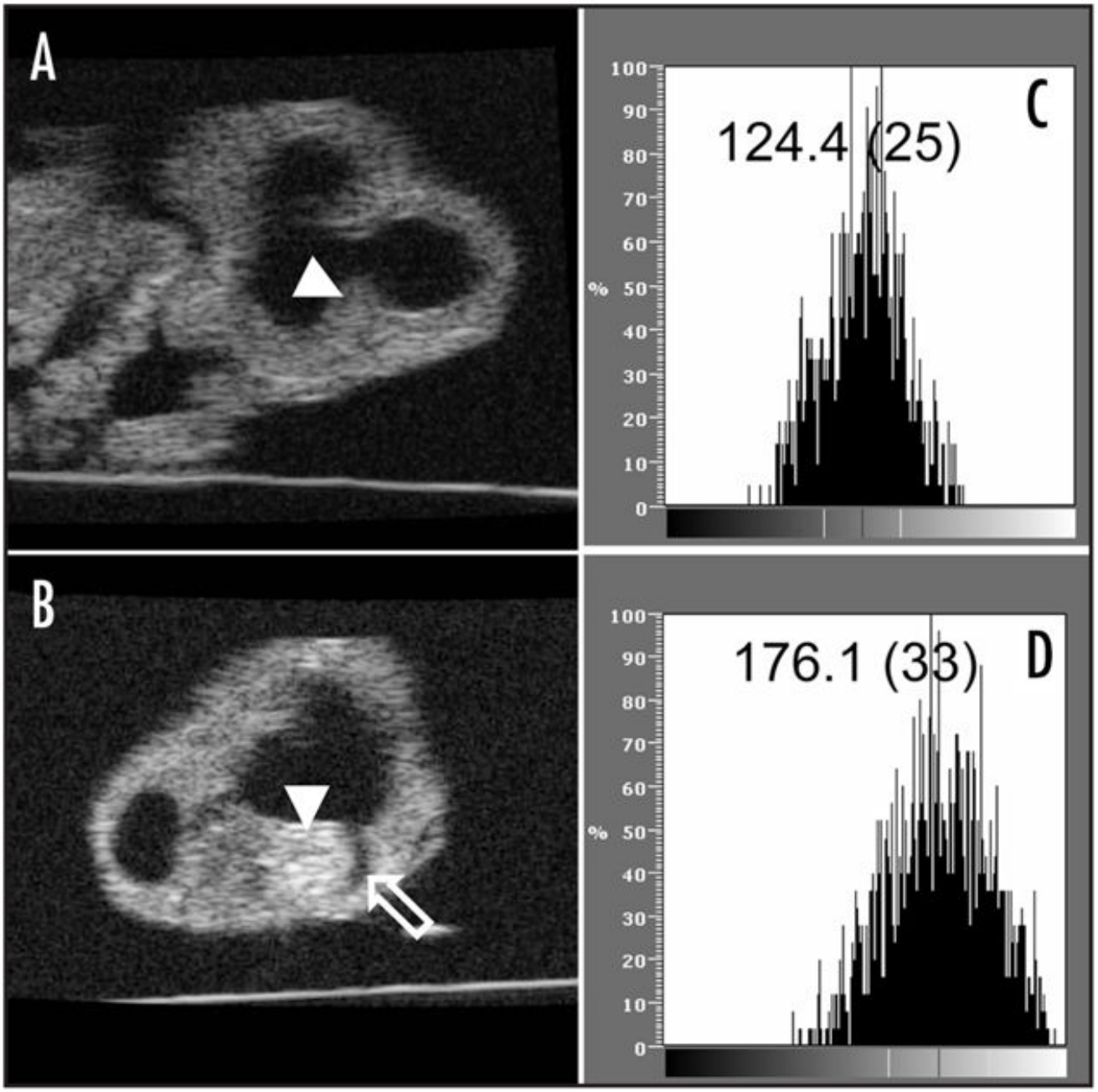

Figure 3.

Ex vivo ultrasound micro-imaging of E10.5 wild-type and DEspR-deficient brains. Ex vivo analysis of fixed E10.5 wild-type (A) and DEspR-deficient (B) embryos by ultrasound micro-imaging using the VisualSonics Vevo770 RMV708 probe (50 micron resolution) detected narrow irregularities (arrow) in the *DEspR*$^{-/-}$ brain (B) compared with the wild-type brain (A). These irregularities are in the primitive telencephalon, most likely the antecedent to the hyperproliferative and convoluted neuroepithelium detected at E12.5 (Figs. 1 and 2). Moreover, quantitative microimaging of wild-type (C) and *DEspR*$^{-/-}$ (D) brains detected areas with increased grayscale intensities (arrowhead) in *DEspR*$^{-/-}$ brain (B) compared to similar region (arrowhead) in wild-type brain (A) and to common reference areas with equivalent

grayscale intensity analyzed at identical settings using Vevo770 integrated software analysis programs (VisualSonics, Inc.,). These areas most likely correspond to increased cellular density in hyperproliferative areas of the neural tube represented here by the telencephalon and diencephalon regions.