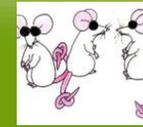


Three Blind Mice

A mutation in ADAM17 is responsible for embryonic eyelid closure defect in woe mice



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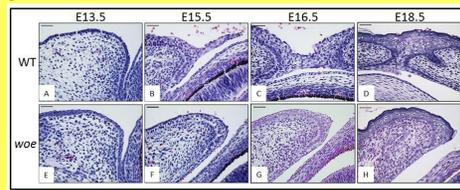
Abstract:

During embryogenesis, in all mammals, the eyelids grow across the eye anterior, fuse together, and subsequently reopen. This process is essential for proper eye development. ADAM17 is a Zn²⁺ metalloprotease that has a role in cleaving numerous proteins including growth factors involved in EGFR signaling, a molecular pathway essential for cell migration. Mice with mutations in genes encoding ADAM17, EGFR, and EGFR ligands exhibit defects in embryonic eyelid closure. Recently, woe (wavy with open eyelids) mice, also exhibiting defects in embryonic eyelid closure, were identified. Genetic analysis of woe mice identified a mutation in ADAM17 leading to three different ADAM17 mutant proteins. Two of these mutant proteins were catalytically inactive; however, the third mutant protein exhibited normal ADAM17 catalytic activity. Further molecular analysis showed that this catalytically active mutant protein exhibited T265M substitution and was expressed at very low levels. The T265 residue is within the ADAM17 Zn²⁺ catalytic domain (215–473 aa). The T265M mutant protein exhibits normal ADAM17 catalytic activity most likely because T265 residue is not in the Zn²⁺ active site. The Saint Joan Antida SMART Team modeled ADAM17's catalytic core using 3-D printing technology. In addition SMART Team identified the location of the T265 amino acid residue within the catalytic core and its position relative to the active site. A better understanding of which amino acids are essential for the ADAM17 catalytic function will ultimately lead to a better understanding of ADAM17-mediated EGFR pathway and the role of this pathway in cell migration and organ development.

Embryonic eyelid closure

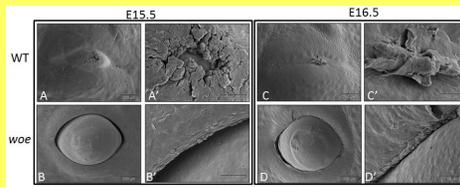
During normal mammalian embryonic development eyelids form, grow, fuse and subsequently reopen. Closed embryonic eyelids serve as a protective barrier by preventing premature exposure of the developing ocular structures to the environment. In mice, the initial step occurs between embryonic days E11.5 and E14.5 when the primitive eyelid is formed. By E15 leading edge begins to form and between E15.5 and E16.0, the leading edge extends and migrates across the eye anterior. The leading edges from both sides of the eyelid root come in close proximity of each other and eventually fuse together by E16.5 (Summarized in Fig. 1 top panel). The eyelids remain fused until ~12 days after birth upon which they reopen. Mice carrying mutations in genes essential for the embryonic eyelid closure are born with open eyelids. For example mice with mutations in genes encoding ADAM17, EGFR, and EGFR ligands exhibit defects in embryonic eyelid closure and die at birth.

Figure 1.



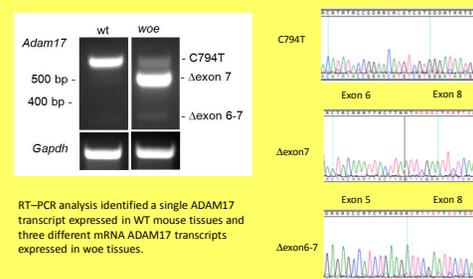
Hematoxylin and Eosin staining of eyelids from wild type (WT) (top) and woe (bottom) mice. At E13.5, no obvious morphological differences were observed between WT (A) and woe (E) mice. At E15.5, the leading edge formed in WT mice (B), however in woe mice the leading edge did not form (F). By E16.5, the leading edges met and formed a junction in WT mice (C) whereas eyelids in woe mice remained open (G). Further histological analysis of woe mice at E18.5 (H) showed the failure of embryonic eyelid closure, in contrast to WT mice that exhibited completed embryonic eyelid closure (D).

Figure 2.



Scanning electron micrographs confirmed absence of the leading edge formation in woe embryos. In E15.5 wild type (WT) mice (A and A'), the formed leading edges were migrating towards each other. In contrast, woe embryos (B) exhibited the absence of even rudimentary leading edges at the rims of the eyelids (B'). By E16.5 in WT embryos (C), the leading edges met, forming the junction (C') whereas in woe mice (D) the eyelids remained open (D').

Figure 3.



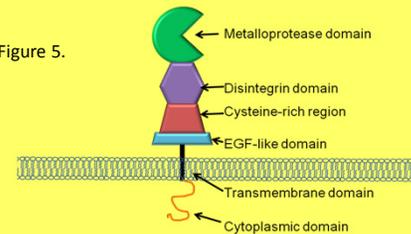
RT-PCR analysis identified a single ADAM17 transcript expressed in WT mouse tissues and three different mRNA ADAM17 transcripts expressed in woe tissues.

Figure 4.



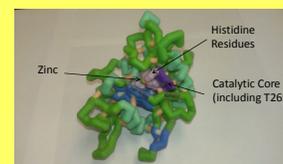
A model of ADAM17 mRNA transcripts identified expressed in wild type mice (top panel) and mutant ADAM17 mRNA transcripts expressed in woe mice (bottom panel). The percentage on the right represents levels of each transcript identified.

Figure 5.



A model of ADAM17 protein depicting domain structures. As a part of the protein sequence ADAM17 also contains an N-terminal signal sequence followed by a pro-domain, which are not depicted in this schematic diagram.

Model of ADAM17 catalytic core



The Saint Joan Antida SMART Team modeled ADAM17's catalytic core using 3-D printing technology. In addition, SMART Team identified the location of the T265 amino acid residue within the catalytic core and its position relative to the active site. The T265M substitution most likely doesn't affect the active site, even though it is located within the catalytic core.

Mutant ADAM17 proteins identified in woe mice

Wild type ADAM17 mRNA transcript encoded by 19 exons leads to an 827 amino acid ADAM17 protein. Mutant ADAM17 mRNA transcripts from woe that lack exon 7 or lack exon 6 and 7 encode catalytically inactive ADAM17 mutant proteins. These two transcripts account for 90% of all ADAM17 mRNA transcripts identified in woe tissue. However, a full length ADAM17 transcript carrying C>T substitution in the nucleotide 794 also identified as expressed in woe tissues encodes a full length ADAM17 protein carrying a T265M substitution. This mutant ADAM17 protein exhibits normal levels of catalytic activity. It should be pointed out that this C794T ADAM17 transcript is present only at 10% of the levels of ADAM17 transcript levels identified as expressed in wild type tissues.

Roles of wild type ADAM17 protein

ADAM17 belongs to a disintegrin and metalloproteases (ADAMs) family of Zn²⁺-dependent proteases that play a role in the proteolytic ectodomain cleavage or "shedding" of membrane-bound proteins. ADAM17 cleaves membrane bound growth factors that transactivate EGFR signaling, a molecular pathway that is essential for cell migration and embryonic eyelid closure. ADAM17 is a multi-domain protein starting with a signal sequence, followed by a prodomain, a metalloprotease or catalytic domain, with the typical HEXXHXXGXXH (X being any amino acid residue) active site sequence, a disintegrin domain, an cysteine-rich domain, followed by a transmembrane domain and a cytoplasmic tail. The catalytic domain is responsible for cleaving membrane bound proteins.

ADAM17 In Conclusion...

A better understanding of which amino acids are essential for the ADAM17 catalytic function will ultimately lead to a better understanding of ADAM17-mediated EGFR pathway and the role of this pathway in cell migration and organ development, specifically in eye development. From these findings a model emerges showing that proteins such as ADAM17 involved in EGFR signaling are essential for cell migration and consequently embryonic eyelid closure. This is different from findings in Drosophila where the EGFR signaling is used reiteratively to trigger the differentiation of each of retinal cell types - successive rounds of EGFR activation lead to formation of photoreceptors, then cone and finally pigment cells.

References

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