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Xenopus ADAM19 is involved in neural, neural crest and muscle development

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ABSTRACT

ADAM19 is a member of the meltrin subfamily of ADAM metalloproteases. In *Xenopus*, ADAM19 is present as a maternal transcript. Zygotic expression starts during gastrulation and is apparent in the dorsal blastopore lip. ADAM19 expression through neurulation and tailbud formation becomes enriched in dorsal structures such as the neural tube, the notochord and the somites. Using morpholino knock-down, we show that a reduction of ADAM19 protein in gastrula stage embryos results in a decrease of Brachyury expression in the notochord concomitant with an increase in the dorsal markers, Goosecoid and Chordin. These changes in gene expression are accompanied by a decrease in phosphorylated AKT, a downstream target of the EGF signaling pathway, and occur while the blastopore closes at the same rate as the control embryos. During neurulation and tailbud formation, ADAM19 knock-down induces a reduction of the neural markers N-tubulin and NRP1 but not Sox2. In the somitic mesoderm, the expression of MLC is also decreased while MyoD is not. ADAM19 knockdown also reduces neural crest markers prior to cell migration. Neural crest induction is also decreased in embryos treated with an EGF receptor inhibitor suggesting that this pathway is necessary for neural crest cell induction. Using targeted knock-down of ADAM19 we show that the reduction of neural and neural crest markers is cell autonomous and that the migration if the cranial neural crest is perturbed. We further show that ADAM19 protein reduction affects somite organization, reduces 12-101 expression and perturbs fibronectin localization at the intersomitic boundary.

1. Introduction

Post-translational modification of proteins is a critical step in most biological processes. Among these modifications, cleavage of cell surface proteins control a diverse range of cellular processes such as cell signaling, cell adhesion and cell migration. One family of metalloproteases, ADAM (protein containing A Disintegrin and A Metalloprotease (Wolfsberg et al., 1995), is responsible for modulating various signaling pathways by shedding membrane bound growth factors and/or their receptors (Blobel, 2005; Huovila et al., 2005; Moss and Lambert, 2002). In addition, ADAMs have also been shown to cleave cell adhesion molecules such as cadherins to promote epithelial to mesenchymal transition (EMT) essential for proper embryonic development (Maretzky et al., 2005; Reiss et al., 2006, 2005).

ADAM19 is a member of the meltrin subfamily and has been shown, in mouse, to cleave TNF-α, Kit1 Ligand, TRANCE,

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HB-EGF and neuregulin-1 (Chesneau et al., 2003; Horiuchi et al., 2005; Kawaguchi et al., 2007; Shirakabe et al., 2001) The knockout (KO) of ADAM19 has been shown to induce defects in heart development, believed to be due to defective HB-EGF signaling (Kurohara et al., 2004; Zhou et al., 2004). Conditional KO of ADAM19 in the cardiac neural crest cells reproduced the heart phenotype suggesting that ADAM19 is critical for cardiac neural crest cell specification and/or function but not migration (Komatsu et al., 2007). In mouse, the main enzyme responsible for the cleavage of both HB-EGF, and neuregulin-1 appears to be ADAM17. In addition, mice lacking ADAM17 show very similar heart defects to ADAM19 KO mice. Furthermore, a double knock out of both ADAM17 and 19 results in a more severe phenotype demonstrating possible functional compensation of these ADAMs during embryonic development (Horiuchi et al., 2005). Fibroblasts derived from ADAM19 KO mice do process both neuregulin-1 and HB-EGF, suggesting that the ADAM19 substrate responsible for its phenotype remains to be identified.

To test whether ADAM19 has a unique role during early embryonic development, we have cloned the *Xenopus laevis* homologue and analyzed its expression pattern. XADAM19 is present at low levels as a maternal transcript with zygotic expression beginning during gastrulation and localizing to the dorsal blastopore lip. During later stages of embryonic development, ADAM19 is expressed in the dorsal mesoderm, neuroectoderm and neural crest cells. Loss of function experiments using antisense morpholino knock-down reveals that the protein is essential for the specification of the dorsal mesoderm, neuronal derivatives and cranial neural crest cells. The apparent lack of functional compensation for ADAM19 in *X. laevis* may help resolve the biologically relevant substrate for ADAM19 during early embryonic development.

2. Results

2.1. ADAM19 is expressed both maternally and zygotically

We have cloned the *X. laevis* homologue of ADAM19 using degenerate oligonucleotides to human, mouse, quail and the genomic sequence of *X. tropicalis*. The full-length sequence was generated by 5′- and 3′-RACE-PCR and assembled from multiple PCR amplicons. The deduced amino acid sequence is 49% identical to human ADAM19 and 84% identical to *X. tropicalis* ADAM19. We found several variants in the cytoplasmic domain at the amino acid level, one of which is most common to the *X. tropicalis* sequence and was submitted to GenBank (EU770696). Amplification using multiple primers from genomic DNA suggests that one intron-less allele is present in the *X. laevis* genome (data not shown).

We investigated ADAM19 expression throughout development using real-time PCR. Since the genomic DNA appears to contain a copy of the gene lacking introns, we used polyA purified mRNA to eliminate any trace of genomic DNA in our samples. ADAM19 is expressed both maternally and zygotically from gastrulation through early tailbud formation (Fig. 1). The expression level of ADAM19 at gastrulation is low but is confirmed by *in situ* hybridization where it appears restricted to the dorsal lip of the blastopore (Fig. 2A).

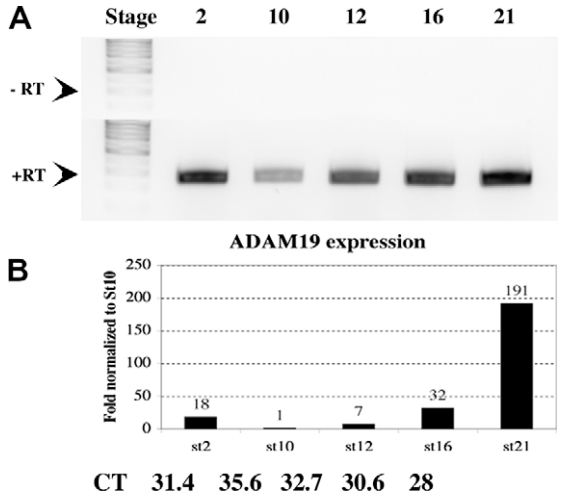

Fig. 1 – ADAM19 expression. Quantitative real-time PCR expression of ADAM19 during early *Xenopus* development. Polyadenylated RNA from 2.5 embryos was used to make cDNA. PCR amplification was performed on a light cycler for 40 cycles. (A) The amplification product from both the control (−RT) and the experimental (+RT) were separated on a 2% agarose gel. (B) The relative abundance of the ADAM19 mRNA was measured by the $2^{(-\Delta C_T)}$ method using stage 10 as a value of 1. Values are not adjusted to a control gene but to identical number of embryos. The CT values for each reaction are given below the graph. No CT values were obtained for the −RT control of each stage. ADAM19 mRNA is present as a maternal transcript at stage 2. Zygotic transcription starts during gastrulation and increases during neurulation (stage 16) continuing into early tailbud stage (stage 21).

2.2. ADAM19 mRNA is enriched in dorsal structures including somites, neural tube and cranial neural crest cells

Using whole mount *in situ* hybridization, we investigated the distribution of ADAM19 transcript during gastrulation, neurulation and tailbud formation (Fig. 2). To avoid background staining due to either antibody or substrate precipitation in the blastocoel, we performed *in situ* hybridization on hemi-gastrula embryos. At stage 12, the mRNA encoding ADAM19 is mostly localized to the dorsal lip of the blastopore where the dorsal mesoderm invaginates (Fig. 2A). During early tailbud stages, ADAM19 is expressed in the dorsal region of the embryo including the neural tissue and the dorsal mesoderm. ADAM19 is detected in the neural tube, the notochord and the cement gland (Fig. 2B, C and F). In the mesoderm, ADAM19 is expressed both in the segmented (somite) and non-segmented (caudal) dorsal mesoderm (Fig. 2G and H). When stained embryos are observed in PBS rather than Murray's clear to visualize superficial staining, ADAM19 is detected in the segments of the cranial neural crest cells (CNC, Fig. 2I). The expression in the CNC cells was confirmed by RT-PCR on explants dissected from stage 17 embryos (Fig. 2I insert). At stage 26, ADAM19 expression is evident in the cement gland, the brain, the somites, the otic vesicle and the branchial arches (Fig. 2D). At stage 36 ADAM19, is still expressed in the entire head, the branchial arches and the