A Novel, Secreted Form of Human ADAM 12 (Meltrin α) Provokes Myogenesis in Vivo*

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The ADAM (A Disintegrin And Metalloprotease) family of cell-surface proteins may have an important role in cellular interactions and in modulating cellular responses. In this report we describe a novel, secreted form of human ADAM 12 (meltrin α), designated ADAM 12-S (S for short), and a larger, membrane-bound form designated ADAM 12-L (L for long form). These two forms arise by alternative splicing of a single gene located on chromosome 10q26. Northern blotting demonstrated that mRNAs of both forms are abundant in human term placenta and are also present in some tumor cell lines. The ADAM 12-L transcript can also be detected in normal human adult skeletal, cardiac, and smooth muscle. Human A204 embryonal rhabdomyosarcoma cells that do not differentiate into muscle cells and do not express any form of ADAM 12 were stably transfected with an ADAM 12-S minigene encoding the disintegrin domain, the cysteine-rich domain, and the unique 94 amino acid carboxyl terminus. Nude mouse tumors derived from these transfected cells contained ectopic muscle cells of apparent mouse origin as shown by species-specific markers. These results may have potential applications in the development of muscle-directed gene and cell therapies.

ADAMs are a recently discovered family of membrane-anchored cell-surface proteins. They are about 800 amino acids long and have a unique domain organization, containing pro-metalloprotease, disintegrin, cysteine-rich, transmembrane, and cytoplasmic domains (1–4). Because these domains are homologous to domains in proteins with established functions, ADAMs have been proposed as candidates for modulating processes. Sequencing of ADAM 12-L (L for long form) expressed in human placental cDNA library (CLONTECH catalog number HL4025AH) us-

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EXPERIMENTAL PROCEDURES

Isolation and Sequencing of Human ADAM 12 cDNA Clones—A positive prey clone (S1) was isolated from a human yeast two-hybrid placental cDNA library (CLONTECH catalog number HL4025AH) us-
ing a cDNA fragment of the lamin β2 chain as bait. This clone was sequenced and found to be similar to mouse ADAM 12. A probe corresponding to the disintegrin domain (nt 1540–1963) was used to screen a human placenta 5′-stretch plus λgt11 DNA library (CLONTECH catalog number HL50146). Twenty-six positive phage were plaque-purified and six were applied to a colony of TALON immobilized metal affinity resin (CLONTECH). After washing the resin with a urea buffer (0.020 M Tris-HCl, pH 7.9, 0.5 M NaCl, 6 M urea, 0.01 M imidazole), elution of bound material was performed with 0.05 M EDTA in 0.020 M Tris-HCl, pH 7.9, and 0.15 M NaCl. The yield from a typical preparation was ~2.5 μg.

Lewis female rats (Møllegaarden, Denmark) and female rabbits (Statens Seruminstitut, Copenhagen, Denmark) were immunized and boosted at monthly intervals with total E. coli fusion protein extract or with purified recombinant ADAM 12 derived from expression construct (p1055) emulsified in complete and incomplete Freund’s adjuvant. Antisera (rab 104) were collected 10–11 days after the second and all subsequent injections. To prepare monoclonal antibodies, rats were immunized and boosted eight times were given a final boost intraperitoneally, and 5 days later hybridomas were prepared by fusing spleen cells from the rat with the nonsecretive mouse myeloma P3 × 63Ag8.653 (ATCC TIB 18) as recently described (32). Supernatants of the resulting hybridomas were screened and characterized for their immunostaining of COS-7 cells transiently transfected with construct number 1095 (see below). The hybridoma 1055 was IgG2b as determined by Ouchterlony immunodiffusion using a series of anti-rat immunoglobulins purchased from Serotec and by the IsoStrip kit from Boehringer Mannheim. Hybridomas were grown in DMEM with Glutamax I and 4500 mg/ml glucose, 1 mM sodium pyruvate, 10 mM HEPES, OPI media supplement (0.15 μg/ml oxaloacetate, 0.05 μg/ml pyruvate, 0.0082 μg/ml bovine insulin (Sigma)). 50 units/ml penicillin, and 50 μg/ml streptomycin and 20% myoclon super plus femail bovine serum (Life Technologies, Inc.) at 37 °C in 10% CO2.

Transfection Assays, Immunostaining, and Immunoblotting—A plasmid for expression of an ADAM 12-S minigene was constructed using the pSecTagB vector (Invitrogen). A DNA fragment coding for the disintegrin domain, cysteine-rich domain, and the unique carboxyl terminal domain of ADAM 12 was excised from p1095 and cloned into the expression vector of pSecTagB yielding a plasmid pSecTagB-Yielding plasmid p1095, consisting of an ADAM 12 minigene driven by a cytomegalovirus promoter and two T7 promoters. Based on an Ig κ-chain leader sequence to allow secretion of the protein. For transient transfections, COS-7 cells were electroporated as described previously (32) with a Bio-Rad Gene Pulser II, using 250 V and 1000 microfarads for 0.4 ml of cells and 10 μg of plasmid in PBS/HEPES, with an electrode gap of 0.4 cm. After electroporation, the cells were plated in Lab-Tek 8-well chambers (Nunc 177402). Cells were transfected with an expression vector of pSecTagB-Yielding plasmid p1095 or the expression vector with no CDNA insert (pSecTagB). Two to three days later, immunostaining was performed as described previously (32). Briefly, the cells were rinsed with PBS, fixed with cold methanol, rinsed with PBS, and incubated with the primary antibodies diluted in 0.05% Tween 20, 0.05 M Tris-HCl, pH 7.2 (1:100 for the polyclonal antibodies and 1:2 with culture medium supernatant of the monoclonal antibody), for 1 h at room temperature. After rinsing, the sections were incubated with fluorescein-conjugated secondary antibodies for 1 h, washed, the slides mounted in buffered glycerol and examined under a Zeiss LSM-10 laser scan microscope.

A204 cells were stably transfected with an expression plasmid for a human ADAM 12-S minigene (p1095) or the expression vector with no CDNA insert (pSecTagB). Cells were transfected with LipofectAMINE from Life Technologies, Inc., using a ratio of 2 μg of DNA to 20 μl of LipofectAMINE. Two days’ post-transfection, the cells were trypsinized and replated in DMEM containing 10% fetal bovine serum and 500 μg/ml Zeocin (Invitrogen). Zeocin-resistant colonies were selected and grown in the presence of 200 μg/ml Zeocin. Clones were assayed for the expression of ADAM 12 by Northern blot analysis of total cellular RNA using ADAM 12 common region cDNA as a probe and for a Southern blotting using ADAM 12-specific antibodies. For detection of ADAM 12 in the medium of transfected cells, confluent cultures of cells were incubated in serum-free UltraDOMA-PF medium (BioWhittaker) for 2 days. The medium was concentrated 10-fold using an Amicon Centricon-10 filter. Samples were subjected to SDS-polyacrylamide gel electrophoresis on 10–20% gradient gels (Novex) and transferred to nitrocellulose.
membranes. The membranes were incubated with medium from 14E3 hybridoma cells or rb 104 polyclonal antiserum and subsequently with peroxidase-conjugated rabbit anti-rat or swine anti-rabbit immunoglobulins (DAKO). Detection was performed using the enhanced chemiluminescence SuperSignal kit from Pierce.

RT-PCR was applied to examine for the presence of the full-length of the human homologue of ADAM 12 transcripts. The human ADAM 12-L sequence with mouse ADAM 12 revealed that it has a structural organization typical for the members of the ADAM family (1), shown schematically in Fig. 1A. Human ADAM-L and -S share a common region consisting of the prodomain (residues 29–206), the metalloprotease domain (residues 207–417), the disintegrin domain (residues 417–512), and the cysteine-rich domain (residues 529–614) that contains the putative fusion peptide. ADAM 12-L has a 21-aa transmembrane domain and a 179-aa cytoplasmic domain. ADAM 12-S has instead a shorter 34-aa carboxyl terminus with no apparent transmembrane domain. Comparison of human ADAM 12-L sequence with mouse ADAM 12 revealed an overall amino acid identity of 81%. Within the individual domains, the sequence similarity to mouse ADAM 12 was high in the cysteine-rich, metalloprotease, and disintegrin domains and lower in the pro- and cytoplasmic domains (Table I). We also compared the ADAM 12 amino acid sequences to all other known ADAMs, and the comparison with the four most similar ADAMs (the Xenopus ADAM 13, human ADAMs 8, 15, and 9) is shown in Table I. The most conserved sequences are in the metalloprotease and the disintegrin domains, and the least conserved regions are the prodomain and the cytoplasmic tail. The divergent carboxyl terminus of ADAM 12-S showed no similarity to any of the other known ADAM proteins nor to any other proteins in the databases.

The human ADAM 12 metalloprotease domain contains the highly conserved zinc-binding motif HEXGHXXGXXH regulated by a potential “cysteine switch” in the prodomain (3). This sequence is identical to mouse ADAM 12 and, as with other ADAMs containing this motif, is presumed to be catalytically active. The disintegrin domain contains a putative integrin binding loop, although like other ADAMs and the related P-III SVMPs, ADAM 12 does not have an RGD sequence (3). Both human and mouse ADAM 12 have the amino acids SNS at this position followed by an additional cysteine residue. The cysteine-rich domain of human ADAM 12 contains the putative fusion peptide-like sequence that can be modeled as a one-sided α-helix with one strongly hydrophobic face (2) and an epidermal growth factor-like repeat (37). ADAM 12-L contains a 21-aa, highly hydrophobic (18/21 aa) transmembrane domain which is consistent with the consensus sequence motif for type I membrane proteins (38). In addition, the flanking amino acid

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sequence is consistent with the amino terminus being exposed to the cell exterior. The cytoplasmic domain of human ADAM 12-L is proline-rich (32 out of 179 aa) and contains at least three sites (RXXPXXP) that are potential ligands for the Src homology 3 domain (SH3) (39), as has been demonstrated for the proline-rich motifs in ADAM 9 (40).

Chromosomal Localization—By using fluorescent in situ hybridization, we mapped the chromosomal localization of the human ADAM 12 gene (ADAM12). 96% of the 30 metaphase cells analyzed showed specific fluorescent spots on the q26 band of the long arm of human chromosome 10 (Fig. 2). Subsequently we searched the data base of mapped STSs on the human genome (41) and identified an STS (WI-17472) that is identical in sequence to part of the 3′-untranslated region of ADAM 12-L (nt 4044–4145). WI-17472 was placed on the distal region of chromosome 10 by radiation hybrid mapping, in the interval between the Genethon markers D10S216 and D10S575 (158–162 centimorgan), consistent with our cytogenetic localization of the gene. Thus the ADAM12 gene is located at 10q26.3.

ADAM 12-L and ADAM 12-S Arise by Differential Splicing—The finding of two cDNA forms of ADAM 12 sharing identical 5′-regions, but diverse 3′-ends, suggested that they were alternatively spliced versions of a single gene. This hypothesis was strengthened by the single chromosomal localization and the observation that a probe for the pro-domain common to both forms of ADAM 12 hybridized to a single band in human high molecular weight genomic DNA digested with seven different restriction enzymes (data not shown). We designed PCR primers to amplify the genomic DNA around the point of divergence in the ADAM 12 clones. Primers 322 and 324 amplified a 4-kb, 325 and 324 a 2-kb, and 322 and 323 a 1-kb DNA fragment (Fig. 3). Sequencing of these revealed that the ADAM 12 gene contains an intron at the point of divergence between the clones, followed by an exon encoding the ADAM 12-S-specific sequence, which does not appear to have any introns within it. At the end of the ADAM 12-S sequence, about 2 kb of intron DNA are present before the ADAM 12-L coding sequence. A consensus 5′-donor site was found at the point of divergence between the ADAM 12-L and -S sequences, and 3′-acceptor sites were present at the start of both the ADAM 12-L- and ADAM 12-S-specific sequences.

Human ADAM 12 mRNA Expression—We examined the expression of ADAM 12 mRNA using probes common to both forms of ADAM 12 and probes specific for each form. Northern blot analysis with a probe for the disintegrin domain present in both forms of human ADAM 12 revealed three bands of 3.5, 5.4, and 8.6 kb in human full-term placenta RNA, expressed at relative levels of 2:1:1 (Fig. 4, lane 1). Probes specific for the ADAM 12-S clone hybridized only to the smallest 3.5-kb band (Fig. 4, lane 3), whereas probes specific for human ADAM 12-L hybridized to the two top bands only (Fig. 4, lane 2). The 3.5- and 5.4-kb bands correspond to the sizes of the full-length ADAM 12-S and -L cDNAs, although we did not isolate a full-length cDNA clone equivalent in size to the 8.6-kb band observed on Northern blots. As the 8.6-kb band hybridized to the same probes as the 5.4-kb ADAM 12-L transcript, this suggests that the 8.6-kb transcript contained the same ADAM 12-L sequence as the 5.4-kb band but had an extended 3′-region. To clarify this further, we searched the dbEST data base with 500 bp of the untranslated region at the 3′ of the

### Table I

| Domains | Amino acid similarity between human ADAM 12 and |
|---------|-----------------------------------------------|
|         | ADAM 12 (mouse) | ADAM 13 (Xenopus) | ADAM 8 (human) | ADAM 9 (human) | ADAM 15 (human) |
| Overall | 84            | 52              | 45            | 45            | 45             |
| Prodomain | 66       | 41              | 38            | 38            | 36             |
| Metalloprotease | 95   | 68              | 57            | 49            | 56             |
| Disintegrin | 91       | 71              | 61            | 35            | 56             |
| Cysteine-rich | 96     | 61              | 46            | 56            | 45             |
| Transmembrane | 95     | 47              | 43            | 35            | 71             |
| Cytoplasmic | 69      | 29              | 20            | 28            | 35             |

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Fig. 1. cDNA cloning of soluble and transmembrane forms of human ADAM 12. A, schematic drawing of the human ADAM 12-L and -S forms of cDNA clones isolated. Selected restriction sites indicated are ApaI (A), BamHI (B), EcoRI (E), HindIII (H), NcoI (N), PstI (P), and XhoI (X). B, the nucleotide and protein sequence of human ADAM 12-L and -S. The ADAM 12-S sequence is only presented from the point of divergence from ADAM 12-L. Matches to the Kozak consensus sequence around the start codon are underlined; potential N-linked glycosylation sites (NX(S/T)) are double underlined; zinc-binding motif (HEGXXXGXXH) is boxed; within the disintegrin loop the underlined SNS aligns with RGD; regions in the cytoplasmic tail matching the SH3 binding site (RXXPXXP) are boxed; and potential polyadenylation signals are underlined, with the polyadenylation site in the slightly smaller ADAM 12-L cDNA clone (L2) indicated at nt 4949. Amino acids that are identical between ADAM 12-L and mouse ADAM 12 (D50411), the partial Xenopus ADAM 12 (43), and the four most similar ADAMS, Xenopus ADAM 13 (U66003), human ADAMs 8 (D26579), 9 (U41766), and 15 (U46005), are indicated in bold italics and those amino acids that are conserved in italics.

Fig. 2. Mapping of the human ADAM 12 gene by in situ hybridization. A, partial metaphase spreads observed after hybridization to a biotinylated human ADAM 12 cDNA probe. Arrows indicate specific hybridization signals detected using fluorescein isothiocyanate-conjugated avidin. B, the same partial metaphase spreads observed after R-banding and staining with propidium iodide to identify the chromosomes. C, ideogram of the human G-banded chromosome 10, showing the localization of the ADAM12 gene at 10q26.2.
ADAM 12-L cDNA clone and were able to assemble a partial contig covering an additional kilobase of 3'-untranslated ADAM 12-L DNA (not shown). Primers specific for this EST-derived region were designed and used to amplify a 467-bp product from placenta cDNA. This probe hybridized only to the 8.6-kb band on a placenta RNA Northern blot (data not shown). Thus the 8.6-kb band observed on Northern blots appears to encode the identical sequence to the ADAM 12-L cDNA but has a longer 3'-untranslated region.

No ADAM 12 transcripts could be detected by Northern blot examination of mRNA from human brain, lung, liver, kidney or pancreas (data not shown). Under the same hybridization conditions expression of the 8.6- and 5.4-kb ADAM 12-L-specific bands was detected in mRNA isolated from heart, prostate, uterus (no endometrium), colon (no mucosa), small intestine, bladder, stomach, and skeletal muscle, but at levels at least 15-fold lower than in placenta (Fig. 4B). The 3.5-kb ADAM 12-S band was not observed in these blots. The source of ADAM 12-L...
mRNA in the uterus, colon, small intestine, bladder, stomach, and prostate may be the smooth muscle cells, a hypothesis that is supported by our preliminary immunohistochemical analysis showing that these cells exhibited a positive immunostaining reaction with anti-ADAM 12 antisera. Northern blot analysis of several cultured human cell lines demonstrated that the RD rhabdomyosarcoma and the HU-1 lung adenocarcinoma cells lines expressed all three ADAM 12 transcripts, although the A204 cell line did not express any (Fig. 4). The ADAM 12 mRNA in these carcinoma cell lines appears to be expressed at a lower level than that observed in placenta but at a higher level than observed in normal tissue.

Some indication of gene expression can be obtained from EST data bases. ESTs specific for human ADAM 12-L have been isolated from cDNA libraries prepared from HeLa cell s, full-term placenta, and 20-week post-conception fetal liver and spleen. ESTs specific for ADAM 12-S have been isolated from cDNA libraries prepared from 6-week embryo, 8–9-week post-conception, and full-term placenta, and 20-week post-conception fetal liver and spleen. This may be taken as evidence that ADAM 12-S is expressed in normal tissues other than placenta.

**Biological Function of ADAM 12**—To begin analyzing the distribution and function of the ADAM 12 protein, we generated poly- and monoclonal antibodies to the 17-kDa cysteine-rich domain of ADAM 12 produced in E. coli (Fig. 5A). These antibodies immunostained and reacted in Western blotting with COS-7 cells transiently transfected with an ADAM 12-S expression plasmid but not with cells transfected with a control plasmid lacking an ADAM 12 insert (Fig. 5, B and C). We then made an expression construct carrying an ADAM 12-S minigene coding for the disintegrin domain, the cysteine-rich domain, and the carboxyl terminus of ADAM 12-S. The rationale for using a minigene was based on previous studies showing that a mouse ADAM 12 minigene lacking the pro- and metalloprotease domains was biologically active, whereas the full-length form was not (25). This plasmid containing the minigene (p1095) or the vector lacking a cDNA insert as a negative control was transfected into the human rhabdomyosarcoma cells A204 that do not express detectable amounts of ADAM 12 mRNA or protein. Three stably transfected clones were obtained that expressed ADAM 12-S minigene mRNA and secreted a 42-kDa ADAM 12-S polypeptide into the medium (Fig. 5D). Like the parental A204 cells, these three cell lines showed no apparent capacity to fuse in vitro (data not shown).

The parental A204 cells and A204 cells transfected with either the ADAM 12-S expression plasmid (three clones) or a control plasmid (three clones) were injected into nude mice and allowed to form subcutaneous tumors. No gross difference in tumor growth capacity was observed. However, morphological analysis revealed a striking difference in the stromal compartment (Figs. 6 and 7 and Table II). The tumors derived from the parental cells and from three control transfected A204 cell lines consisted of densely packed tumor cells with an appearance consistent with embryonal rhabdomyosarcoma. In contrast, in tumors generated by three ADAM 12-S minigene transfected A204 cell lines, a striking, bizarre pattern of muscle cell differentiation was observed (Fig. 6A). Irregular stellate and elongated myocyte- and myotube-like cells were scattered randomly in the stroma. The nuclei of the myotubes were either centrally or peripherally located, and cross-striation was seen in some of

* R. Albrechtsen and U. M. Wewer, unpublished observations.
Table II

Effect of overexpression of the ADAM 12-S minigene on de novo myogenesis in A204 embryonal rhabdomyosarcoma cells in nude mice

| Cell type injected | No. of tumors | No. of tumors with muscle differentiation |
|--------------------|---------------|------------------------------------------|
| Controls           |               |                                          |
| A204 parental      | 21            | 1                                        |
| A204-mock          | 14            | 2                                        |
| Total              | 35            | 3 (8.6%)                                 |
| ADAM 12-S transfected |             |                                          |
| A204-1095-7        | 14            | 13                                       |
| A204-1095-10       | 2             | 2                                        |
| A204-1095-15       | 14            | 12                                       |
| Total              | 30            | 27 (90%)                                 |

FIG. 7. Characterization of the ectopic muscle cells in nude mouse tumors generated by A204 cells transfected with the ADAM 12-S minigene. Immunostaining was performed with antibodies to the following markers: A, tetranectin; B, myogenin; C, caveolin-3; D, mouse laminin-1; E, human laminin β1; F, p53. Symbols used are: T, tumor cells; N, normal adult muscle fibers in cross-section; arrows, ectopic muscle cells; open arrowsheads, tumor nuclei with positive immunostaining. Scale bars: A, 10 μm; B, 20 μm; C–E, 50 μm; F, 20 μm.
5.4- and 8.6-kb transcripts appear to be derived by alternative use of polyadenylation sites in the ADAM 12-L-specific 3′-untranslated region. We have isolated the full-length cDNA representing the 5.4-kb transcript, but the 8.6-kb transcript appears to contain a longer 3′-untranslated region has not been entirely isolated. The different 3′-untranslated regions of ADAM 12-L could affect the rates of translation or mRNA stability (50).

Analysis of the distribution patterns of the two ADAM 12 forms in normal human tissues revealed that the ADAM 12-S transcript was detected so far only in placenta, whereas the ADAM 12-L mRNAs were found in placenta and skeletal, cardiac, and smooth muscle. Splicing of the ADAM 12-S exon may be regulated by cell type-specific factors. The human ADAM 12-L transcript appears to have a more widespread expression than the mouse mRNA (25). Interestingly, both forms were detected in some tumor cell lines, indicating a possible association between ADAM 12-S and neoplasia.

Until now only two ADAMs that lack a transmembrane domain, and thus are assumed to be secreted, have been reported. These are ADAM 11/MDC-524 (18) and ADAMTS-1 (ADAM with thrombospondin motifs (12)). To this list we now add ADAM 12-S, and we have shown that it becomes secreted. The candidate tumor suppressor MDC-524 splice form was isolated from human cerebellar cDNA library and is expressed at very low levels compared with the cell membrane anchored form MDC-769 (18). ADAMTS-1, which is associated with cancer cachexia and inflammatory processes, lacks the cysteine-rich, transmembrane, and cytoplasmic domains, having instead a thrombospondin homologous domain and type I thrombospondin motifs (12). Like ADAM 12-S, these cancer-related, secreted ADAMs appear to have very restricted, low levels of expression in normal tissue. The best characterized soluble ADAM-like proteins are the snake venom metalloproteases (SVMPs) (7). The soluble ADAMs lacking the regulatory control of a transmembrane domain may be extremely potent like their SVMP counterparts. The highly restricted and low level of normal expression of these soluble ADAMs may reflect this potency, and continued comparison of ADAMs with SVMPs is warranted.

Mouse ADAM 12 has been implicated in cell fusion during C2C12 differentiation in vitro (25). In the present study we found that cells transfected with the shorter, secreted form of ADAM 12-S appear to be very potent in provoking myogenesis in vivo. We stably transfected the human embryonal rhabdomyosarcoma cell line A204 with the ADAM 12-S minigene composed of the disintegrin and cysteine-rich domains and the unique carboxyl terminus. Although a potential muscle precursor, the A204 rhabdomyosarcoma does not differentiate in vitro either spontaneously or after transfection with the ADAM 12-S minigene. However, nude mice tumors generated from these ADAM 12-S minigene transfected cells contained a striking pattern of ectopic muscle cell formation as compared with control tumors. A mixture of cells representing different stages of normal myogenesis was observed, including myoblasts and elongated multinucleated myotubes with cross-striation. These developing muscle cells were located in a disorganized pattern, as opposed to the normal adult skin muscle. Electron microscopy and immunostaining confirmed that these cells were in fact of the muscle cell lineage. Furthermore, based on combined immunostaining using mAbs specific for mouse and human antigens and RT-PCR using species-specific primers for myf-5, we conclude that these ectopic muscle cells are of an apparent murine origin rather than derived directly from the A204 human tumor cells.

What is the cell of origin for this myogenesis? There are at least two possibilities; one is the satellite cells, which are normally located in intimate relationship with existing myofibers beneath the basement membrane. Satellite cells are ubiquitous in normal adult muscle and represent the muscle progenitor cells during muscle regeneration (24). Another possible source is undifferentiated mesenchymal progenitor cells present in connective tissue. The mechanism by which ADAM 12-S may be involved in the recruitment and differentiation of muscle progenitor cells is not clear, and how it acts in the context of factors from the A204 rhabdomyosarcoma cells and/or the host stroma remains to be resolved.

In conclusion, we have characterized a novel form of secreted human ADAM 12, designated ADAM 12-S, and presented evidence that it provokes myogenesis in a nude mouse tumor model.

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