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# Soluble Form of Jagged1: Unique Product of Epithelial Keratinocytes and a Regulator of Keratinocyte Differentiation

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**Abstract** Notch signaling pathway is an important regulator of epithelial differentiation. Recent studies have characterized multiple ligands, including Jagged1, as mediators of Notch signaling. In this work, an alternatively spliced transcript of Jagged1 was isolated in yeast two-hybrid screening through interaction with thrombospondin-1. This transcript, devoid of sequences encoding the transmembrane and intracellular domains of Jagged1, was specific for keratinocytes. Furthermore, the truncated Jagged1 polypeptide devoid of the intracellular domain was detected prominently in the suprabasal keratinocyte layer in neonatal epithelia. The soluble form of Jagged1, when expressed as a tagged polypeptide was efficiently secreted into the culture medium and the N-terminal signal sequence became cleaved off upon secretion. Direct contact between a cell presenting the transmembrane form of Jagged1 and a Notch-expressing cell has been proposed as a prerequisite for the signaling event. However, the soluble form of Jagged1, when present in the cell culture medium, was sufficient to induce keratinocyte differentiation. These observations suggest a novel mechanism of how Jagged1 can target cells beyond the direct cell–cell contact in developing epithelia. J. Cell. Biochem. 92: 1271–1281, 2004. 

**Key words:** soluble Jagged1; thrombospondin-1; keratinocytes; epidermis; differentiation

The epidermis at the surface of the skin and the stratified epithelium of the mucosal membranes provide major protection for the body against the outside environment [Nemes and Steinert, 1999]. The stratified epithelium is a constantly self-renewing tissue. In the basal cell layer of epidermis, the balance between proliferating keratinocytes and the proportion of keratinocytes directed to terminal differentiation is crucial to the maintenance of the normal structure and function of the skin [Lehrer et al., 1998; Jensen et al., 1999; Janes et al., 2002; Potten and Booth, 2002; Watt, 2002]. Epidermis is connected to the underlying dermis through the basement membrane zone, which separates the two compartments [Burgeson and Christiano, 1997; Moll and Moll, 1998]. Extracellular matrix in dermis is synthesized and secreted by dermal fibroblasts. Multiple signaling pathways and soluble factors regulate the normal homeostasis of these structures.

The Notch signaling pathway, an important mediator of cell fate selection [Artavanis-Tsakonas et al., 1999] is also a key regulator of keratinocyte proliferation, growth arrest, and entry into differentiation [Lowell et al., 2000; Rangarajan et al., 2001; Nickoloff et al., 2002]. Jagged1 was originally isolated as a mammalian ligand that activates Notch1 [Lindsell et al., 1995]. The activation domain has been mapped to the N-terminal extracellular region of Jagged1, as a specific peptide from the Delta/Serrate/Lag2 (DSL)-domain of Jagged1 that can mediate the activation of Notch signaling, inducing complete maturation of human keratinocytes through NF-κB and PPARγ [Nickoloff et al., 2002]. Notch and its ligands are integral transmembrane proteins and generally transmit signals only between cells in direct contact

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[Lewis, 1998]. An alternatively spliced transcript encoding Jagged1, devoid of the transmembrane and intracellular domains, has been isolated from cDNA library screening [Zimrin et al., 1996]. The expression of the soluble extracellular domain of Jagged1 has been shown in fibroblasts to repress the function of its transmembrane form and to induce the formation of the Src-dependent chord-like phenotype [Wong et al., 2000; Small et al., 2001]. The overexpression of soluble Jagged1 can cause decreased cell–matrix adhesion, suggesting that soluble Jagged1 may have a functional role in tissue remodeling [Lindner et al., 2001]. While Notch activation suppresses fibroblast growth factor (FGF)-dependent cellular transformation leading to the anchorage independent growth of NIH-3T3 fibroblasts [Small et al., 2003], the suppression of Notch signaling by both soluble Jagged1 and soluble Delta1 is mediated through the FGF receptor signaling [Small et al., 2003; Trifonova et al., 2004]. The secreted forms of Delta and Serrate, the two known *Drosophila* Notch ligands, mimic loss-of-function mutations in the Notch pathway [Sun and Artavanis-Tsakonas, 1997], and the truncated forms of the corresponding lin-12/Notch family ligands in *Caenorhabditis elegans* have intrinsic signaling activity [Fitzgerald and Greenwald, 1995].

Here, an alternatively spliced transcript encoding the soluble form of Jagged1 was shown to be prominently present in keratinocytes. Because the soluble form of Jagged1 has a potential to target surrounding cells beyond the direct cell–cell contact, it may also regulate cell–matrix adhesion through interacting with TSP1.

## MATERIALS AND METHODS

### Expression Constructs

For the two-hybrid bait constructs, a cDNA-clone encoding TSP1, aa 400–563, was inserted into pGB-MEL1 and the yeast two-hybrid screening executed as described before [Aho and Uitto, 1998].

A cDNA-clone encoding the soluble form of Jagged1 was produced by PCR with a forward primer 5′-GCC ACC ATG GAC TAC AGG GAC GAC GAT GAC AAG GGT ATG CGT TCC CCA CGG ACG-3′, where the sequence encoding the FLAG-tag epitope, preceded by an optimized Kozak sequence, is followed by a sequence encoding Jagged1 aa 1–6 (underlined), and a reverse primer 5′-ATC ATA GGC TAG CGT AAT CCG GAA CAT CGT ATG GGT ACT TTG TTC TGT TCT TCA GAG GCC-3′, encoding Jagged1 aa 1125–1131 (underlined), joined to the sequence encoding the HA-tag, followed by a translation stop codon. Jagged1 amino acids are given according to the Jagged1 sequence in the GenBank (accession no. AF003837). A mixture of the first-strand cDNAs from placenta, pancreas, and liver (Human multiple tissue cDNA panels, BD Biosciences Clontech, Palo Alto, CA) was used as a template for PCR reaction with DyNAzyme EXT DNA polymerase (Finnzymes OY, MJ Research, Inc., Watertown, MA), and the PCR reaction was executed according to the manufacturer's instructions. A 3.2-kb PCR-product was first ligated into an *Eco*RV-cut and T-tailed pcDNA3 vector. After screening for a clone in correct orientation, the Jagged1 cDNA was released with *Hin*dIII-*Not*I digestion and ligated to *Hin*dIII-*Not*I digested pCEP4 (Invitrogen, Carlsbad, CA), which had been modified to drive the expression of the transgene from a minimal cytomegalovirus (CMV) promoter under the control of seven repeats of a tetracycline response element. The resulting cloning vector, pCEP4t, also contains the *EBNA-1* gene for autonomous replication in mammalian cells and a hygromycin gene for the selection of transfected cells.

Competent JM109 *Escherichia coli* cells (Promega, Madison, WI) were used for transformations, and plasmids were purified using Wizard Miniprep Kit (Promega). Purified plasmids were sequenced to confirm the inframe ligation of the inserts.

### Analysis of Jagged1 Transcripts

Human multiple tissue cDNA panels (human panels I and II, and human fetal panel) were obtained from Clontech and used as templates for PCR analysis. A tenfold serial dilution of a human keratinocyte cDNA library DNA, the first-strand cDNAs produced by reverse transcriptase from RNA of HaCaT cells and a collection of squamous cell carcinoma RNA were also used as PCR templates. Jagged1 primers (p561) 5′-GAA ACA GCT CGC TGA TTG CT and (p562) 5′-AGT GGT TCA GTA TTA TGT ACG A, produced a PCR fragment 1.72 kb from the transcript encoding a transmembrane form of Jagged1 and a PCR fragment 0.22 kb from the

alternatively spliced transcript encoding a soluble form of Jagged1. PCR conditions were: 2 min at 95°C, followed by 38 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min. PCR was conducted using Taq DNA polymerase and the Q-solution provided with the kit (Qiagen, Valencia, CA). G3PDH-primers, provided by Clontech with each MTC panel, were used in a control reaction, and PCR was performed for 30 s at 94°C, followed by 26 cycles at 94°C for 20 s, and 68°C for 2 min. The PCR-products were separated on 1.5% agarose–TBE gels.

### Cell Cultures

MDCK Tet-Off cell line was purchased from Clontech and grown according to the provider's instructions. For the transfections, cells were plated on 60-mm plates, and FuGENE$^{TM}$ 6 Transfection Reagent (Roche Diagnostics, Indianapolis, IN) was used in the ratio of 1 μg of DNA to 3 μl transfection reagent, following the manufacturer's instructions. Doxycycline was included in the transfection medium, and 24 h after transfection, selection was started with hygromycin. Cultures were trypsinized and replated before reaching confluency, usually every 3–4 days. After 3–5 passages, there were no more adherent cells in the untransfected control plates. For the induction of the transgene expression, cells were plated after trypsinization without doxycycline, let grow to 70% confluency, trypsinized again, and replated on 35-mm tissue culture plates to be harvested for Western blotting and into the 4-well chamber slides to be analyzed through indirect immunofluorescence (IIF). To produce soluble Jagged1-containing spent medium for keratinocyte cultures, Jagged1-MDCK cells grown either with or without doxycycline were let to reach 70% confluency in T-75 flasks, and defined KGM (Clonetics, San Diego, CA), with or without doxycycline and supplemented with 0.15 mM $Ca^{2+}$, was applied on the cells. The spent media were filter-sterilized through the 0.2 μm filters (Millipore, Bedford, MA) and aliquoted for storage at −70°C.

Human foreskin keratinocytes were isolated by cell culture core unit in the Department of Dermatology in Thomas Jefferson University. Keratinocytes at passage 1 were used for the experiments described here. After separating from epidermis with trypsin treatment, keratinocytes were plated on Matrigel-coated 8-well chamber slides and let to adhere and reach 50% confluency. The spent MDCK-cell medium was applied on the keratinocytes for 3 days before preparing the slides for IIF.

### Antibodies

Following commercially available primary antibodies were used: goat anti-Jagged1, specific for Jagged1 N-terminus (N-19, 1:500, Santa Cruz Biotechnology, Santa Cruz, CA, sc-6012), rabbit anti-Jagged1, specific for Jagged1 C-terminus (H-114, 1:500, Santa Cruz Antibodies, sc-8303), rabbit anti-FLAG (1:500, AbCam, Cambridge, MA), mouse monoclonal anti-FLAG M2 (1:2000, Stratagene, La Jolla, CA), rabbit anti-HA-tag (1:3000, Covance, Richmond, CA), mouse monoclonal anti-HA-tag (1:5000, Covance), rabbit anti-desmoplakin (1:5000, Serotech Ltd., Raleigh, NC), monoclonal anti-keratin10 and anti-keratin14 (1:1000, Neomarkers, Fremont, CA), anti-actin mAb (1:5000; Boehringer/Roche), anti-involucrin mAb (1:2000; Neomarkers). Rabbit antiserum for a peptide at periplakin head domain was produced and purified as described earlier [Kazerounian and Aho, 2003; Aho, 2004]. Secondary antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). The HRP-conjugated anti-mouse antibody was used for Western blotting in 1:25,000. Texas Red- and FITC-conjugated species-specific secondary antibodies were used for IIF in 1:500 dilution in PBS–1% BSA. Nuclei were visualized with DAPI, which was included in the secondary antibody incubation.

### Immunoblotting

For Western blotting, cells in each 35-mm culture dish were washed three times with cold PBS and lysed in 0.1 ml of SDS-sample buffer (BioRad, Hercules, CA), supplemented with β-mercaptoethanol. After boiling for 10 min, 10 μl aliquots of cell lysates were separated on 4–20% acrylamide–SDS gels. After transfer onto PVDF-membrane, the membranes were blocked for 1 h at RT in PBS–1% BSA 5% non-fat dried milk powder. After the primary antibody incubation overnight at 8°C, the membranes were washed four times, 10 min each wash, in TBS–0.5% Tween 20, incubated for 1 h at RT with the secondary antibody, and washed again, and the signal was developed using Western Lightning$^{TM}$ Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, Boston, MA).

### Immunofluorescence Studies

For the IIF, tissues were embedded in the OCT-compound, frozen, and cut into 7-μm sections. Slides were either stored frozen at −20°C or used immediately. Tissue sections as well as the cultured cells on the chamber slides were fixed with methanol for 10 min and, after two washes with PBS, further permeabilized with 0.1% Triton X-100 in PBS for 5 min, followed by three washes with PBS. Blocking was done with 1% BSA in PBS for an h at RT, followed by the primary antibody incubation at 8°C overnight. Slides were washed three times with PBS, each time for 5 min, followed by the secondary antibody incubation at RT for 1 h. After three washes with PBS, slides were mounted and studied under a fluorescent microscope (Axioskop, Carl Zeiss, Inc., Thornwood, NY). The images were stored with ImagePro Plus 4.0 imaging software (Media Cybernetics) and processed with Photoshop 5.0 (Adobe Systems, Inc.) and Canvas 5 (Deneba Software).

## RESULTS

### Interaction of Thrombospondin-1 With the Non-Transmembrane Form of Jagged1

The extracellular matrix can greatly influence the biology of the cells in direct contact. Thrombospondin-1 is known as a versatile matricellular protein, present in developing and regenerating tissues [Bornstein, 1995]. To identify proteins that interact with TSP1, a yeast two-hybrid screen was carried out with the central region of TSP1, aa 450–559, as a bait. In order to focus specifically on the proteins secreted by keratinocytes, a human foreskin keratinocyte cDNA expression library was selected as a target. A strong interaction was identified with a partial cDNA clone, encoding human Jagged1 (Fig. 1). Further DNA-sequencing revealed that the isolated cDNA clone encodes Jagged1 aa 742–1065, including the epidermal growth factor (EGF)-repeats 9–16 and the cystein-arginine-rich (CR)-domain. However, sequences encoding the transmembrane and intracellular domains of Jagged1 were not present, but sequences encoding the extracellular juxtamembrane domain joined directly to the middle of the 3-untranslated region (UTR). This cryptic splicing event deletes 1.5 kb from the transcript, but the Jagged1 polypeptide becomes truncated only by 151 aa, from 1218 to 1067 aa residues.

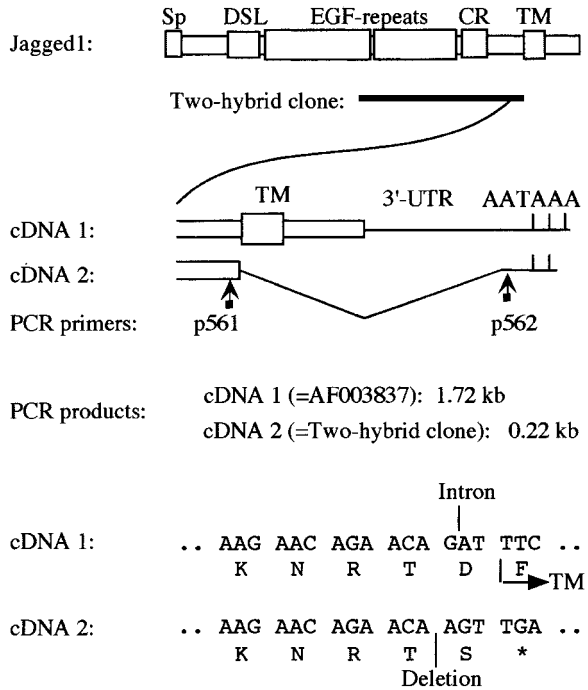


**Fig. 1.** A transcript encoding the non-transmembrane form of Jagged1 is generated through alternative splicing. The Jagged1 polypeptide is composed of distinct domains of signal peptide (Sp), Delta/Serrate/Lag-2-like domain (DSL), epidermal growth factor (EGF)-like repeats, cysteine-arginine-rich domain (CR), and a transmembrane domain (TM). Two-hybrid screening of keratinocyte library with TSP1 (aa 450–559) as a bait identified Jagged1 as an interacting protein. DNA sequencing revealed that the two-hybrid clone is a partial cDNA clone encoding the extracellular domain of Jagged1, aa 572–1066, joined to nt 5154 within the 3′-untranslated region (UTR), and extending up to nt 5636 between the second and third AATAAA-signals (according to GenBank #AF003837). Two primers, p561 and p562, were designed to distinguish the transcript encoding the transmembrane form of Jagged1 (PCR-product 1.72 kb) from the transcript encoding the soluble form of Jagged1, devoid of the transmembrane and intracellular domains (PCR-product 0.22 kb). The shortened cDNA encodes a protein with a C-terminal serine preceding the translation stop codon, truncating the full-length Jagged1 by 151 aa, from 1218 to 1067 aa residues.

Human multiple tissue Northern blots, often probed with the Jagged1 3′-UTR, have consistently revealed from various tissues only a single band at about 6 kb [Li et al., 1997; Oda et al., 1997; Gray et al., 1999]. However, those blots do not include RNA from skin or epidermal keratinocytes. To specifically address the status of Jagged1 3′-end, primers complementary to the sequences flanking the deletion site (p561 and p562; Fig. 1) were used for the PCR-amplification of the multiple tissue cDNA panels (Fig. 2). A 1.72-kb PCR-product, corresponding

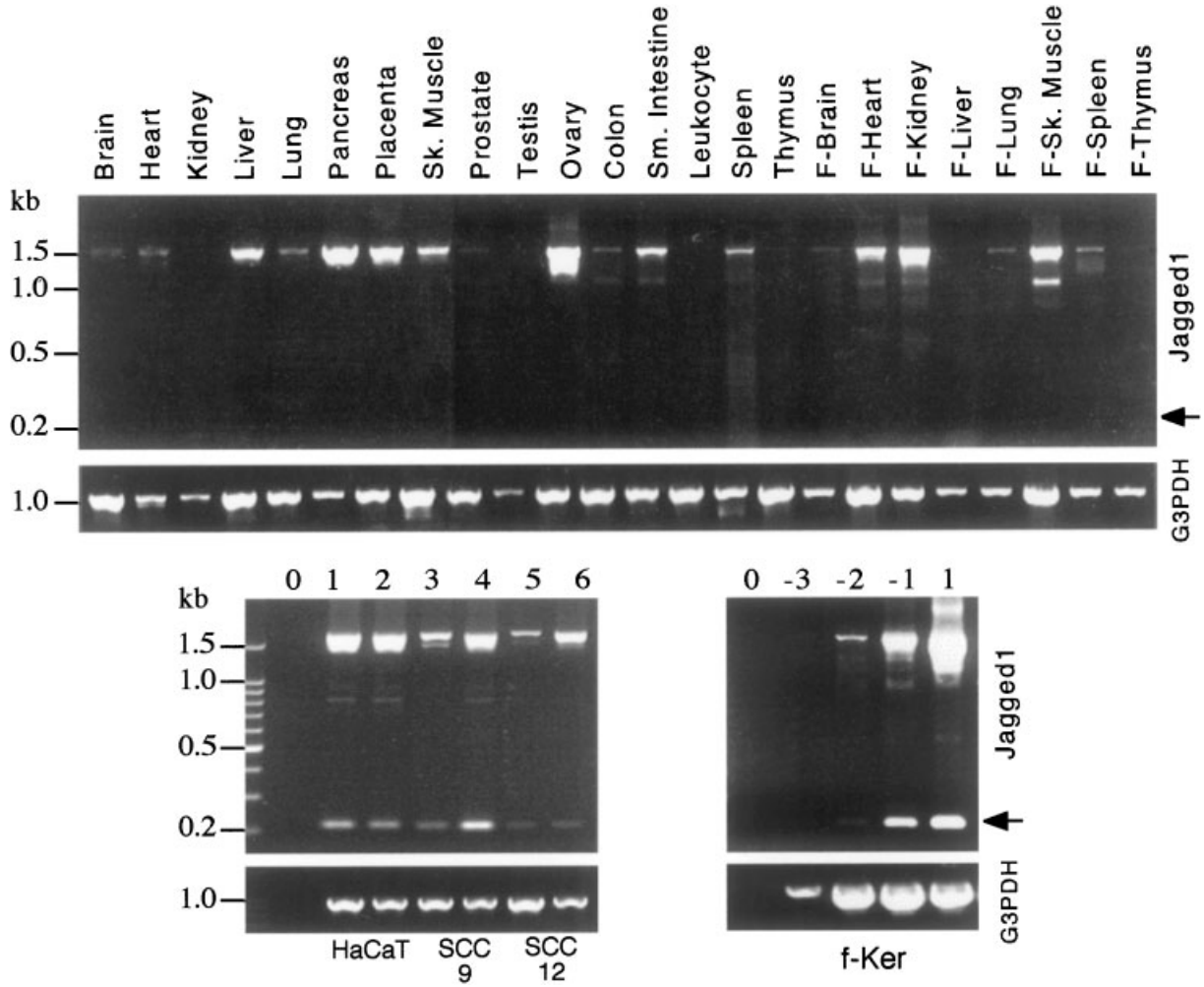


**Fig. 2.** Tissue-specific expression of Jagged1 transcripts. Human multiple tissue cDNA panels I and II (lanes brain to thymus), the cDNA panel of fetal tissues (lanes F-brain to F-thymus), reverse transcriptase products of RNA isolated from HaCaT cells, squamous cell carcinoma lines SCC9 and SCC12, and a serial dilution of human keratinocyte (f-ker) cDNA-library DNA (**lane 1**, 1 μg; **lane −1**, 0.1 μg; **lane −2**, 0.01 μg; **lane −3**, 0.001 μg; **lane 0**, no template) were used as templates for the PCR-amplification with Jagged1-specific primers p561 and p562 (shown in Fig. 1), which produced a 1.72-kb fragment from the transcript encoding the transmembrane form of Jagged1, and a 0.22-kb fragment (indicated by an arrow) from the transcript encoding the shortened soluble form of Jagged1. The short PCR-product representing the soluble Jagged1 transcript was obtained only from keratinocytes and squamous cell carcinomas.

to the full-length transmembrane form of Jagged1, was obtained from adult liver, pancreas, placenta, skeletal muscle, and ovary, and from fetal heart, kidney, and skeletal muscle, which is in agreement with the Northern blot results, described above. However, when human keratinocyte cDNA was used as a template for PCR, in addition to the 1.72-kb band, a strong 220-bp product was obtained. The short PCR product was also obtained from HaCaT cells, an immortalized but nontumorigenic keratinocyte cell line, and from two squamous cell carcinoma lines (SCC), indicating that the transcript encoding the non-transmembrane form of Jagged1 is characteristic for epidermal keratinocytes.

### Expression of Soluble Jagged1 by a Specific Subpopulation of Epidermal Keratinocytes

An antibody specific for the amino-terminal domain of Jagged1 revealed a previously undetected expression pattern for the C-terminally truncated soluble Jagged1 (Fig. 3). In neonatal human and mouse epidermis, soluble Jagged1 was abundantly expressed by the suprabasal keratinocytes in immediate contact with the basal cell layer. However, soluble Jagged1 was hardly detectable in the cryosections of adult

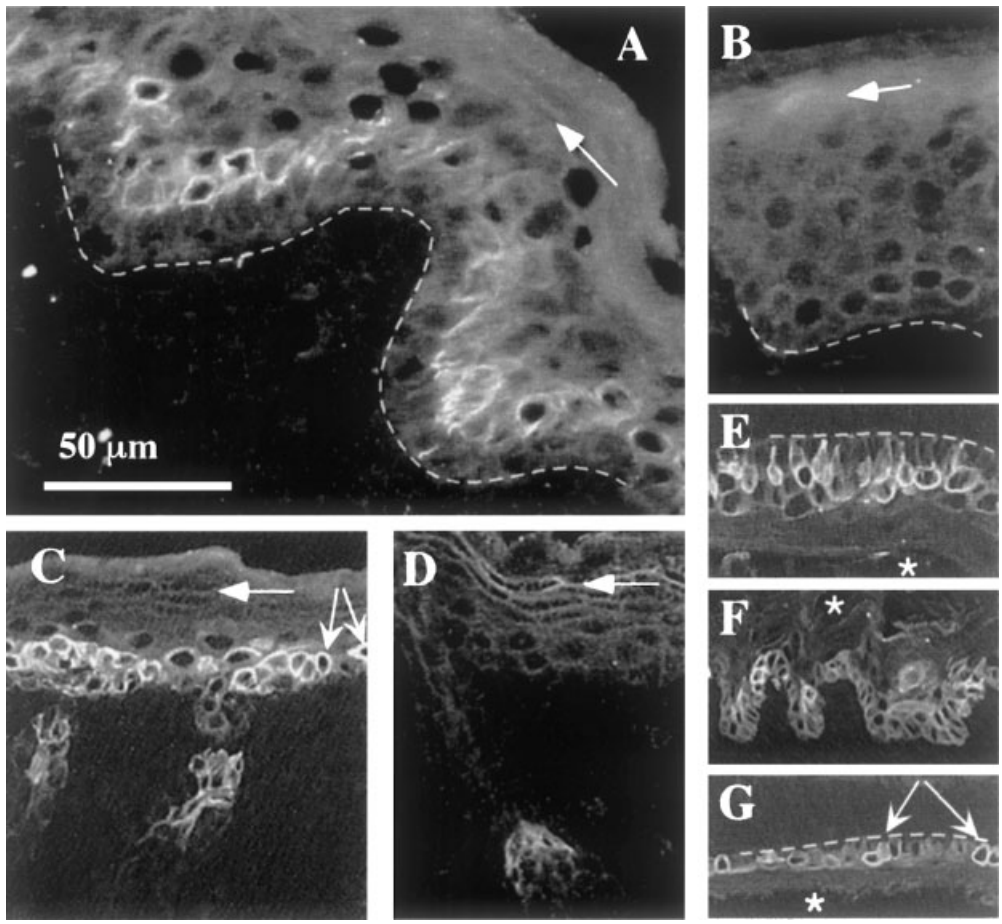


**Fig. 3.** Soluble Jagged1 is present in neonatal human (**A**) and mouse (**C**) epidermis, and in the epithelia of palate and tongue (**E**–**G**), while the suprabasal staining was not present in adult epidermis (**B**). The transmembrane form of Jagged1, detected with the C-terminus-specific antibody is more prominently present in the outer layers of epidermis (pointed by arrows) (**D**). The dermal–epidermal junction is highlighted with a dashed line. The scale bar in A applies to all images. The oral cavity is indicated by an asterisk in E, F, and G. Double arrows in C and G point to the strongly positive cells.

human skin, which suggests a developmentally controlled expression for the soluble form of Jagged1. Further examination of the neonatal mouse palatal and tongue epithelia revealed that in the stratified mucosal epithelium, soluble Jagged1 was present in the suprabasal keratinocytes. However, within the single layer of epithelial cells in the ventral side of tongue, an occasional cell was devoid of Jagged1. Although the nuclei were clearly negative, the positive signal may localize to cytoplasm as well as to the immediate extracellular space.

### Differential Detection of Tagged Jagged1 With Domain-Specific Antibodies

In order to address the functional role of the soluble Jagged1, an expression construct encoding Jagged1, from the initiation codon ATG up to the transmembrane domain, was prepared and equipped with an N-terminal FLAG-tag and a C-terminal hemagglutinin (HA)-tag (Fig. 4A). This construct, under a tetracycline regulatable promoter, was introduced into the tetracycline transactivator dependent (tTA)-Madin Darby Canine Kidney (tet-Off MDCK) cell line. To induce the expression of the transgene, cells were trypsinized and replated in the medium without doxycycline. IIF revealed a population of cells strongly staining with both the antibody specific for the Jagged1 N-terminus and the HA-tag antibody (Fig. 4C). However, as expected, the signal sequence at the amino terminus became almost quantitatively processed when the primary translation product was directed to the secretory pathway and consequently, only an occasional cell stained positive with the FLAG-tag antibody (Fig. 4B).

Western blotting of Jagged1 MDCK cells, cultured with or without doxycycline, confirmed that the transgene was not expressed in the

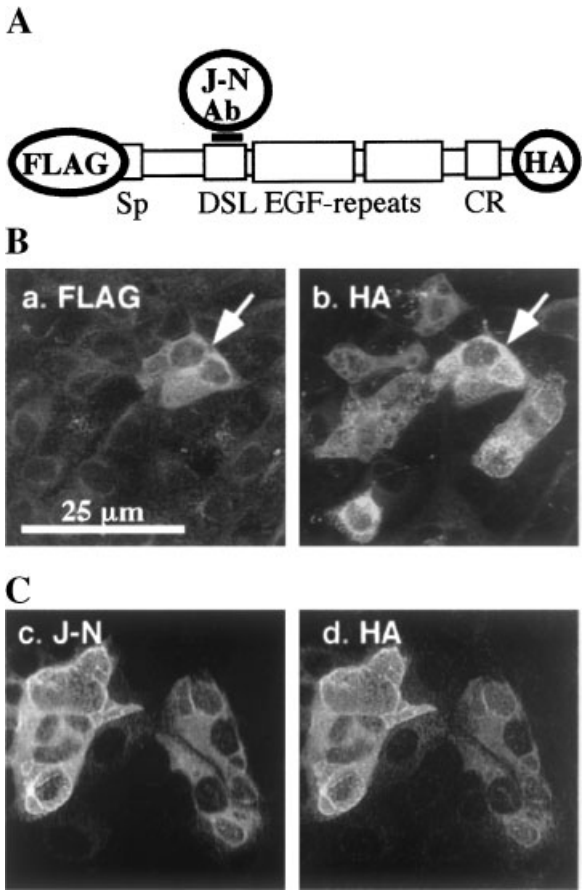


**Fig. 4.** Expression of the tagged soluble Jagged1. The sequences encoding an N-terminal FLAG-tag and a C-terminal HA-tag were incorporated into the PCR-primers, when generating the expression construct encoding the soluble Jagged1 (**A**). MDCK-cells expressing the transgene were tested through indirect immunofluorescence (IIF). **B**: Double-labeling with antibodies specific for FLAG-tag (**a**) and HA-tag (**b**) revealed that the majority of cells expressed the HA-tagged protein, but only a few cells were detectable with the FLAG-tag antibody, suggesting a rapid processing of the secretory signal peptide from the N-terminus. **C**: Signals obtained with the Jagged1 N-terminus-specific antibody J-N (**c**) and HA-tag antibody (**d**) were equally strong and also demonstrated overlapping staining pattern, indicating that these antibodies recognized the same polypeptide.

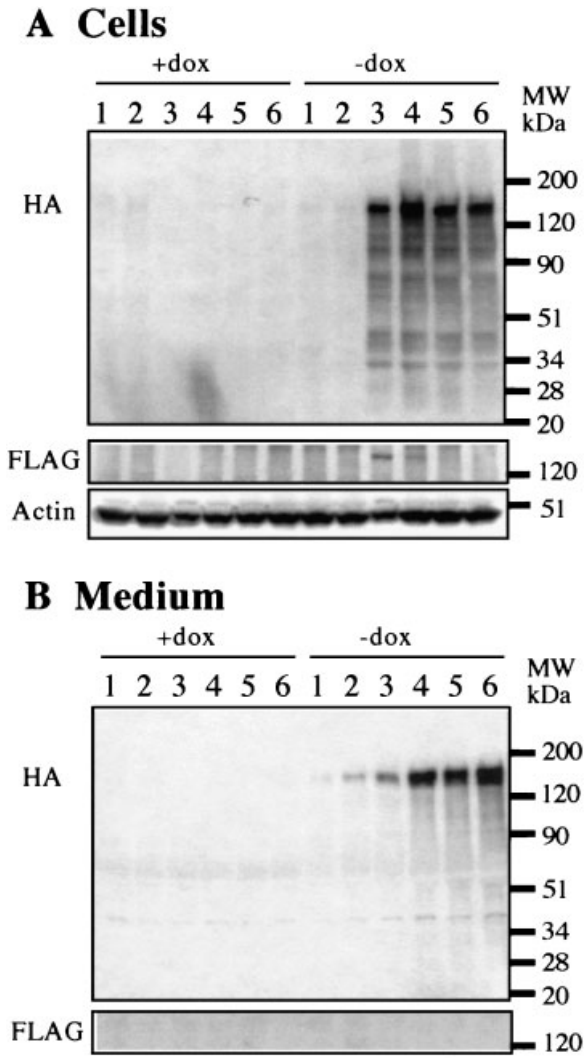


**Fig. 5.** The non-transmembrane form of Jagged1 becomes secreted into the culture medium. Jagged1 MDCK Tet-Off cells were grown with or without doxycycline to subconfluent cultures, trypsinized, and divided into multiple parallel cultures. One plate of each culture was harvested at days 1–6 after plating. HA-antibody demonstrates the accumulation of the C-terminally tagged Jagged1 both in the cell layer (**A**) and in the culture medium (**B**) in a doxycycline regulatable manner, but the FLAG-tag antibody reveals a weak signal in the cell layer at day 3 after plating the cells without doxycycline (A), which is not detectable in the culture medium (B). The HA-antibody detected degradation products of the Jagged1 protein in the cell layer, but the secreted Jagged1 was remarkably stable in the culture medium. For the Western blotting, cells were grown in 35-mm dishes, washed, and lysed directly into 100 μl of the SDS-sample buffer, and a 10 μl (1/10) aliquot was loaded into each well (A). From the spent medium (total of 2 ml) a 5 μl aliquot (1/400), mixed with the Laemmli SB and boiled, was applied into each well. Actin staining is shown (A) as a loading control for both, Jagged1 expressing and non-expressing cells.

presence of doxycycline (Fig. 5). After omitting doxycycline from the culture medium, cells were passaged once to induce the expression of the transgene. In the cell layer, the HA-tag antibody staining revealed Jagged1 degradation products, while the intact soluble Jagged1 peptide was abundantly secreted into the culture medium. Western analysis also confirmed that Jagged1 polypeptide becomes efficiently directed to the secretory pathway as the FLAG-tagged N-terminus with the secretory signal sequence was processed almost quantitatively.

The FLAG antibody revealed a weak signal in the cell layer at day 3, about the time when the Jagged1 production has just started, but as expected, the unprocessed molecule was not detectable in the culture medium (Fig. 5).

### Soluble Jagged1-Mediated Induction of Differentiation Markers in Keratinocytes

Undifferentiated primary foreskin keratinocytes in culture were exposed to the soluble Jagged1 for 3 days, and the expression of differentiation-specific markers was studied through IIF (Fig. 6). Keratin14 is known to be expressed by undifferentiated keratinocytes in culture and by the basal cell layer in the epidermis. Exposure to Jagged1 did not change the keratin14 staining pattern in the basal cells, but a differentiated stratified keratinocyte colony in Jagged1-treated culture was found to be negative for keratin14, although double-labeling revealed a strong signal for desmoplakin (Fig. 6A). In the keratinocyte culture, an occasional cell stained positive for keratin10, while in the Jagged1-treated culture, large flat cell sheets stained positive for keratin10 (Fig. 6B). Desmoplakin, a marker of desmosomes, was found in the Jagged1-treated cultures to be redistributed along the cell–cell junctions and also between the stratified layers of differentiated keratinocytes (Fig. 6A,B). Periplakin and involucrin, markers of differentiated keratinocytes, were each abundantly present in the Jagged1-treated keratinocytes, and colocalized to the cell–cell junctions, especially to the apical plasma membrane of the stratified keratinocyte layers (Fig. 6C). Furthermore, the redistribution and upregulation of keratinocyte differentiation markers by soluble Jagged1 was obtained in the low calcium medium, while the synthetic Jagged1 peptide needed 2 mM calcium to induce epidermal maturation [Nickoloff et al., 2002].

## DISCUSSION

This work demonstrates that an alternatively spliced transcript encoding the soluble form of Jagged1 is an endogenous product of epidermal keratinocytes. In the neonatal epidermis, the expression of soluble Jagged1 at the interface between the basal cell layer and the terminally differentiated keratinocytes is prominent and, in contrast to that of the transmembrane form of Jagged1, which has been reported to be present throughout the epidermis [Nickoloff et al., 2002].

Alternative splicing is a regulatory mechanism affecting the structure and consequently, the functional properties of many proteins. Distinct domains, such as intracellular, extracellular, or transmembrane domain, if omitted, affect the subcellular localization of the corresponding polypeptide. Commonly, N- or C-terminal sequences are used to raise peptide antibodies. The epitope masking is a well-known problem in the immunohistochemistry. However, in the case of soluble Jagged1, where the alternative splicing event eliminates the C-terminal sequences, the truncated protein will not be detected by the C-terminus-specific antibody. Consequently, it is not a surprise that the expression pattern, detected in this work for the soluble Jagged1 differs from what has been reported for the transmembrane form of Jagged1 in human epidermis [Nickoloff et al., 2002].

Proteolytic processing is another mechanism to show how cells modulate functionality of proteins. Both Notch and its ligand Delta are proteolytically cleaved. The entire extracellular domain of Delta has been found as a soluble product, released by the ADAM disintegrin metalloprotease Kuzbanian [Qi et al., 1999; Mishra-Gorur et al., 2002]. Loss of Kuzbanian function results in *Drosophila* phenotypes that are similar to those associated with the loss of Notch signaling, suggesting that Delta cleavage is an important down-regulating event in Notch signaling. In addition, Kuzbanian is required for the cleavage of Notch [Lieber et al., 2002]. The cleaved intracellular domain enters the nucleus and activates the transcription. The role of proteolytic processing in the generation of soluble Jagged1, as well as the role of alternative splicing in generating the soluble Delta, is not known.

Nuclear factor κB (NF-κB) plays a central role in epidermal biology [Kaufman and Fuchs, 2000]. The expression of Jagged1 is under the control of the NF-κB signaling pathway [Bash et al., 1999]. On the other hand, complete keratinocyte differentiation was induced through activation of NF-κB pathway by a Jagged1 peptide [Nickoloff et al., 2002]. Thus, soluble Jagged1, similar to a Jagged1 peptide, may induce keratinocyte differentiation through NF-κB pathway. The results presented in this work show that the soluble Jagged1

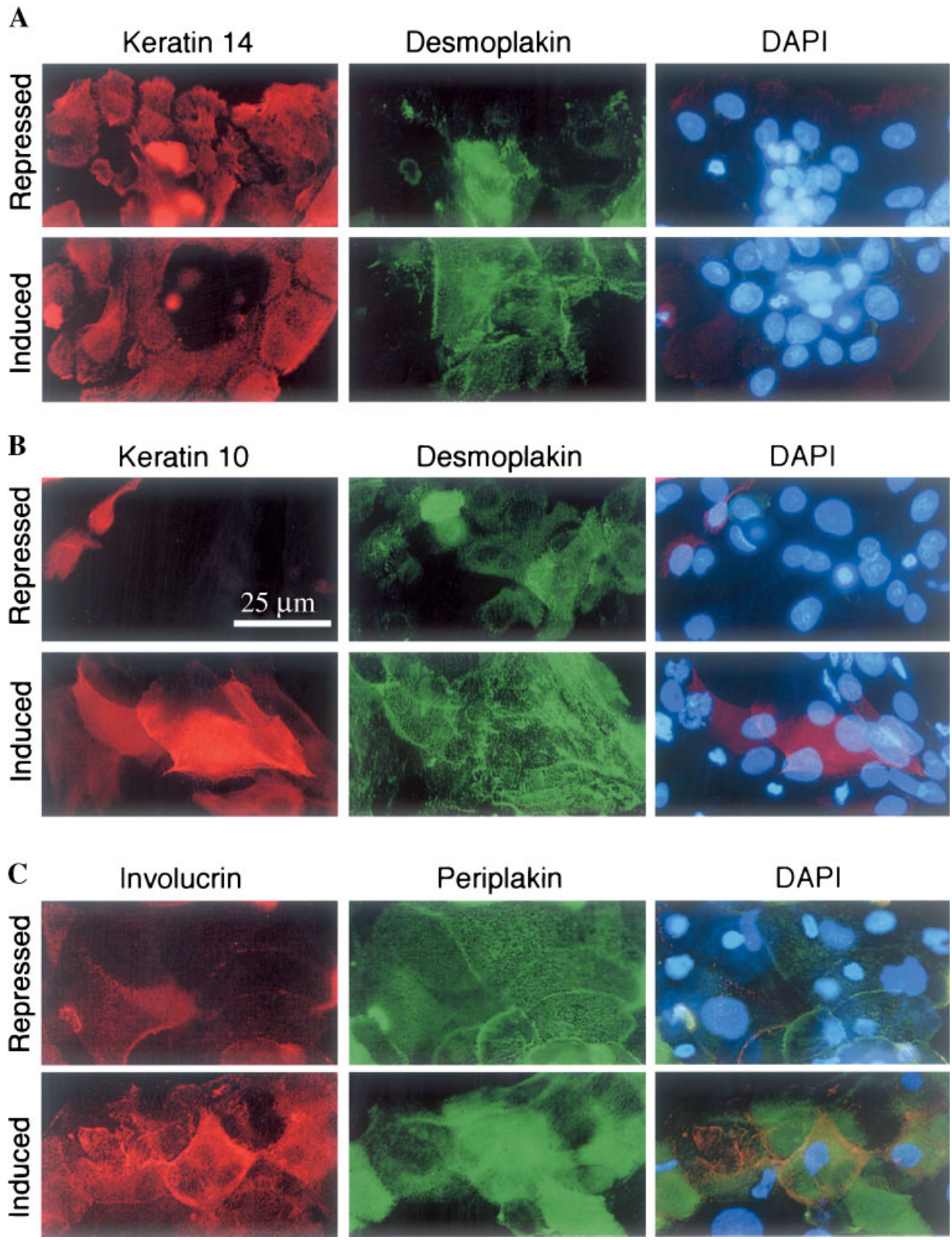


**Fig. 6.** Exposure to soluble Jagged1 directs keratinocytes towards differentiated phenotype. Human neonatal foreskin keratinocytes, seeded into the chamber slides, were treated for 3 days with the spent KGM medium from MDCK-cells, with (induced) or without (repressed) soluble Jagged1. Cells were prepared for IIF with antibodies specific for keratin14, keratin10, desmoplakin, involucrin, and periplakin. Double labeling for keratin14 and desmoplakin (**A**) demonstrated abundant keratin14 in the basal cell layer, while a cluster of cells exposed to soluble Jagged1 had lost keratin14, but showed increased desmoplakin expression. The upregulation of both keratin10 and desmoplakin (**B**), and involucrin and periplakin (**C**), indicates that soluble Jagged1 in the culture medium is sufficient for the induction of keratinocyte differentiation.

induced keratinocyte differentiation. However, HaCaT cells, when exposed to the soluble Jagged1, did not respond through upregulating the differentiation-specific proteins (data not shown). It has been found that the NF-κB pathway is constitutively active in HaCaT cells [Chaturvedi et al., 1999], which may render these cell unresponsive to soluble Jagged1.

Notch receptors play a key role in several cellular processes including proliferation and differentiation. Keratinocyte-specific deletion of the *Notch1* gene has been shown to result in epidermal hyperplasia and deregulated expression of multiple differentiation markers [Rangarajan et al., 2001]. Although Jagged1 mediates the activation of Notch, thus promoting the non-transformed cellular phenotype, the soluble Jagged1 has been found to antagonize this function and promote the transformed phenotype in NIH 3T3 fibroblasts [Small et al., 2001]. In contrast, an exogenous Jagged1 peptide has been shown to induce keratinocyte differentiation [Nickoloff et al., 2002], which is in agreement with the results in this work. Thus it appears that the soluble Jagged1, while promoting fibroblast cell proliferation, directs epithelial cells towards the differentiated phenotype. The molecular mechanisms underlying these opposite effects are not known. Different response and requirements for the growth factors, such as fibroblasts responding to the FGF and epithelial cells depending on the EGF, may explain the controversial observations between these two cell types.

The localization pattern in neonatal epithelia indicates that soluble Jagged1 has a potential to influence the cells underneath the basal cell layer, especially in the case of the single cell layer epithelium. The stable expression of the extracellular soluble Jagged1 protein in NIH3T3 cells results in the repression of pro-α2(I) collagen transcript and the pro-α1(I) collagen translation products [Wong et al., 2000] and in decreased cell–matrix adhesion and cell migration defects [Lindner et al., 2001]. Furthermore, high Jagged1 expression at the regenerating wound edge [Lindner et al., 2001] indicates a physiological role for the interaction between Jagged1 and TSP1.

As a conclusion (Fig. 7), the alternative splicing event regulates the synthesis of a cell-bound and a secreted form of Jagged1. In this work, the transcript, encoding the soluble form of Jagged1, as well as the corresponding protein, were shown to be specific for epidermal keratinocytes. The mechanism by which the soluble Jagged1 regulates the keratinocyte differentiation remains to be elucidated. Although soluble Jagged1 is unable to contribute to those signaling pathways, where the intracellular PDZ-domain of Jagged1 is involved [Ascano et al., 2003], the soluble form of Jagged1 can function as a Notch ligand and has a potential to target neighboring cells beyond the immediate cell–cell contact. Furthermore, the role for soluble Jagged1 in the extracellular matrix, highlighted through the interaction with thrombospondin-1, is currently not known.

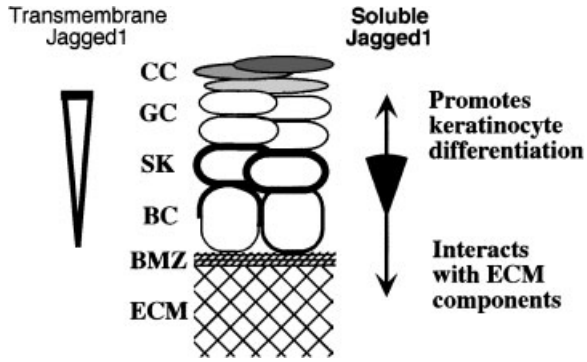


**Fig. 7.** Transmembrane form and the soluble form of Jagged1 in the epidermis. While the transmembrane form of Jagged1 is expressed throughout the epithelium (triangle on the **left side**), soluble Jagged1 is most abundant in the suprabasal keratinocyte layer (arrowhead on the **right side**). As a soluble molecule, the non-transmembrane form of Jagged1 can extend its effect up to the granular cell layer and down to the extracellular matrix of dermis. CC, cornified cell layer; GC, granular cell layer; SK, suprabasal keratinocytes; BC, basal cell layer; BMZ, basement membrane zone; ECM, extracellular matrix.

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