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Notch Ligands

Introduction

The highly conserved Notch signaling pathway regulates many different cell fate decisions in both vertebrate and invertebrate species [1-4]. It is important for pattern formation during development such as neurogenesis, angiogenesis or myogenesis and regulates T cell development and stem cell maintenance [5-7]. But Notch signaling is also involved in cellular processes throughout adulthood [8]. Signaling via Notch occurs between neighbouring cells and both the receptor and its ligands are transmembrane proteins (see Figure 1).

Notch ligands are single-pass transmembrane proteins with a DSL (Delta, Serrate, LAG-2)-domain and varying numbers of EGF-like repeats. There are two classes of canonical Notch ligands, the Delta/Delta-like and the Serrate/Jagged class. The later has an additional domain of cysteine rich repeats close to the transmembrane domain. There are 5 canonical Notch ligands in mammals: Jagged-1, Jagged-2, DLL1, DLL3 and DLL4. These can bind to the four Notch receptors Notch 1-4.

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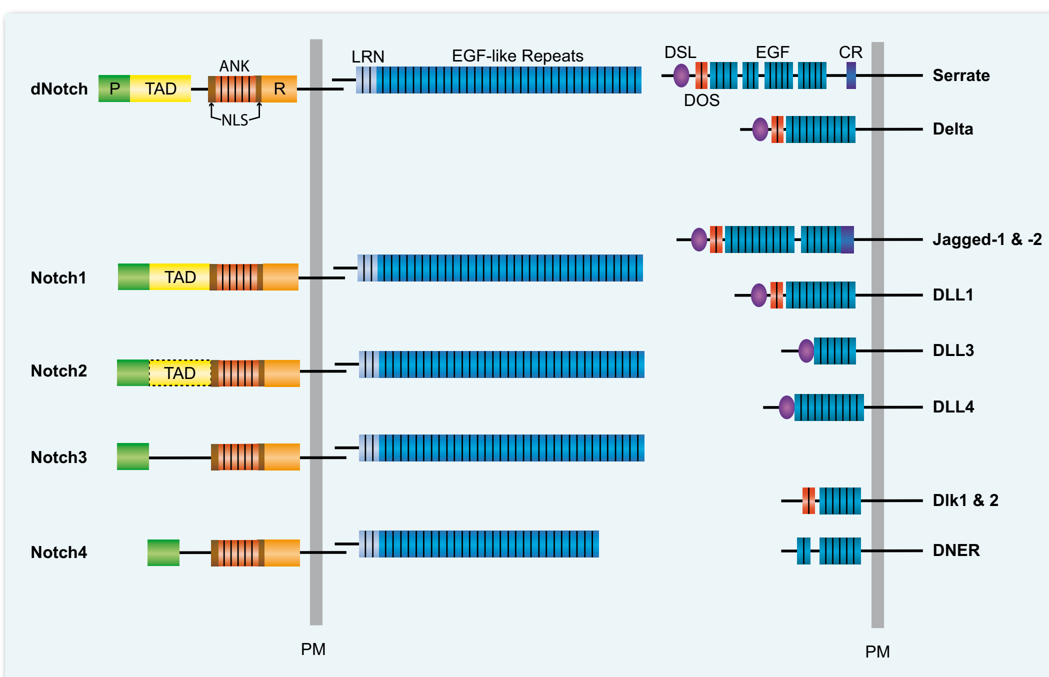

FIGURE 1: Notch Receptors and Their Ligands.

Drosophila contains one Notch receptor (dNotch) that is bound by two transmembrane DSL-ligands (Delta and Serrate). Mammals possess four Notch receptors (Notch1–4) and five ligands (Jagged-1 and -2, which are homologous to Serrate, and Delta-like (DLL) 1, 3 and 4, which are homologous to Delta). Additional noncanonical Notch ligands are Dlk1, Dlk2 and DNER.

ANK: ankyrin repeats, CR: cysteine-rich domain, DOS: Delta and OSM-11-like proteins domain DSL: Delta, Serrate and LAG-2 domain, EGF: epidermal growth factor-like repeats, LNR: cysteine-rich Lin12-Notch repeats, NLS: nuclear localization signal, P: PEST domain, PM: plasma membrane, R: RAM domain, TAD: transactivation domain. Adapted from: *The intracellular region of Notch ligands: does the tail make the difference?* A. Pintar, et al.; Biol. Direct **2**, 19 (2007), *The canonical Notch signaling pathway: unfolding the activation mechanism:* R. Kopan & M. X. Ilagan; Cell **137**, 216 (2009)

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In contrast to mammals, *Drosophila* has only two Notch ligands - Delta and Serrate - that can activate the single Notch receptor [9-11].

The noncanonical Notch ligands lack the DSL domain, among these are Dlk1, Dlk2 and DNER [12-15]. Other noncanonical ligands lack all typical Notch ligand domains and can have a completely different structure, some are not even membrane-tethered [16]. They are thought to act as co-ligands to enhance or inhibit Notch activation and might be important modulators of the Notch pathway [14, 16].

Endocytosis of Notch ligands in the signal-producing cells is absolutely required for the initiation of Notch signaling. Two structurally distinct E3 ubiquitin ligases, Neuralized (Neur) and Mind bomb (Mib), are known to regulate the endocytosis of Notch ligands in Drosophila and zebrafish, respectively [42, 43]. In mammals, 2 Neur homologs, Neur1 [44, 45] and Neur2 [46], and 2 Mib homologs, Mind bomb-1 (Mib1) [47] and Mib2 [48], have been identified. Although all 4 E3 ubiquitin ligases are known to induce the endocytosis of Notch ligands *in vitro,* only Mib1 has an obligatory role in the activation of Jag- as well as DLL-mediated Notch signaling in mammalian development, while Neur1, Neur2, and Mib2 are dispensable [49].

Upon ligand binding, the Notch receptor is cleaved proteolytically (see Figure 2). This cleavage occurs in a two step-process releasing the extracellular and the intracellular part of Notch from the membrane. First the extracellular domain with the bound ligand attached to it is released by the action of a ADAM family metalloprotease creating a membrane-bound intermediate called Notch extracellular truncation (NEXT) (S2 cleavage). In a second step, NEXT is cleaved within the transmembrane domain by γ-secretase (S3/S4 cleavage), releasing the Notch intracellular domain (NICD). NICD translocates to the nucleus and with the help of additional enhancers and co-activators activates target genes [10, 17, 18].

Each Notch molecule signals only once without signal amplification by second messengers. This allows a rapid and highly responsive downregulation and reactivation of the signaling pathway. The activity of Notch and its ligands requires endocytosis and is regulated through glycosylation, ubiquitinylation and microRNAs by mechanisms not yet fully understood [19-27].

The Notch pathway plays an important role in many different processes in a wide range of tissues, this is why aberrations in Notch signaling components have been associated with various human disorders such as cancer, immune disorders, developmental syndromes, stroke and cognitive symptoms [10, 28-36]. A mutation in JAG1 can cause the Alagille syndrome [37-39] and other cognitive dysfunctions such as CADASIL or schizophrenia have been associated with mutations of the Notch receptors [40, 41].

FOR LITERATURE REFERENCES SEE PAGE 4

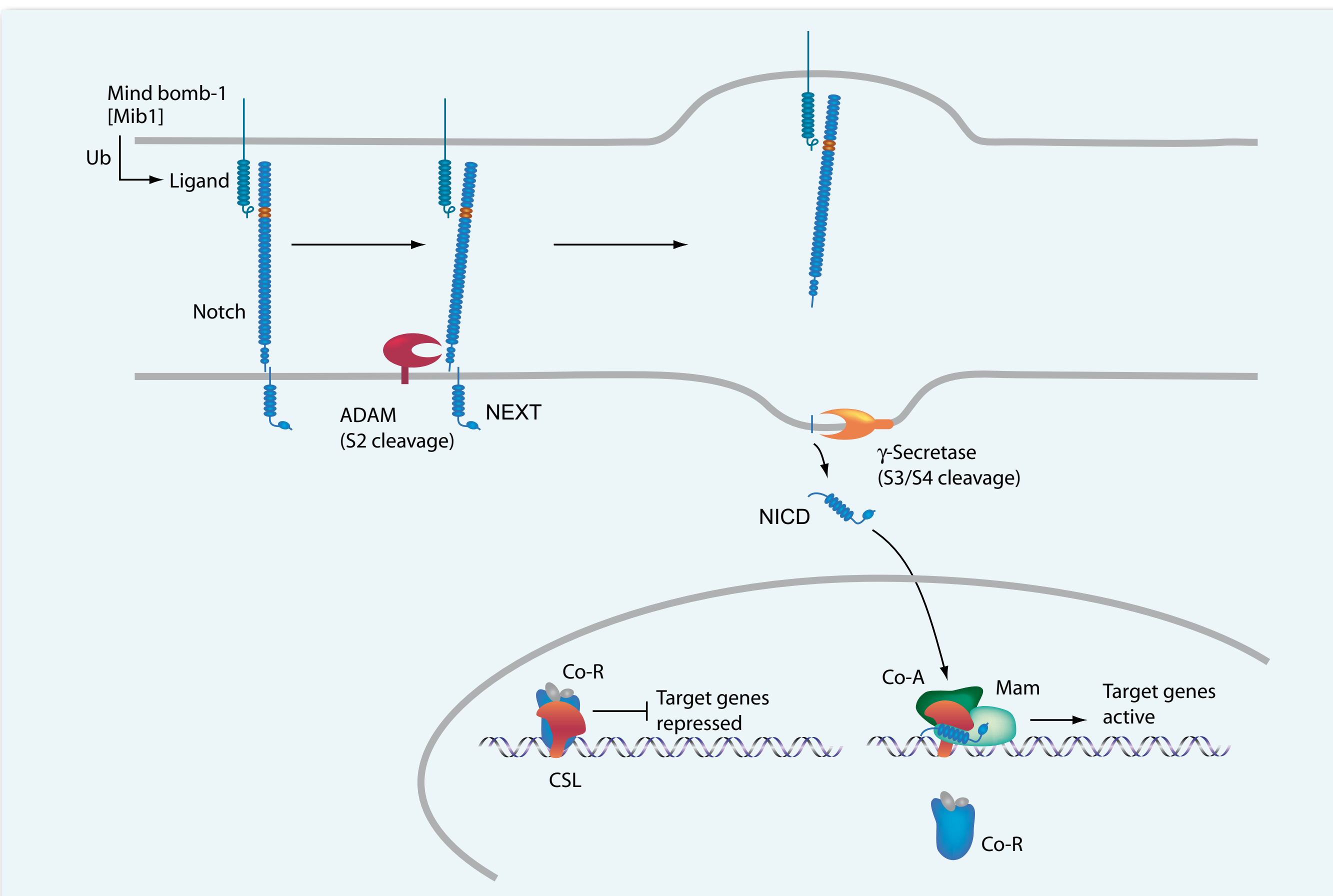

FIGURE 2: The Notch Pathway.

Binding of the Notch ligand (green) on one cell to the Notch receptor (blue) on another cell results in two proteolytic cleavages of the receptor. First a protease of the ADAM family cleaves off most of the extracellular part of Notch leaving a Notch extracellular truncation (NEXT) (S2 cleavage). Second γ-secretase cleaves NEXT at positions S3 and S4 within the transmembrane domain releasing the Notch intracellular domain (NICD). NICD enters the nucleus and interacts with the DNA-binding CSL protein (red), Mastermind (Mam; green) and additional co-activators (Co-A; green). Co-repressors (Co-R; blue and grey) are released and target genes become active.

Adapted from: *Notch signalling: a simple pathway becomes complex:* S. J. Bray; Nat. Rev. Mol. Cell. Biol. **7**, 678 (2006). *The canonical Notch signaling pathway: unfolding the activation mechanism:* R. Kopan & M. X. Ilagan; Cell **137**, 216 (2009)

DLL1 [Delta-like Protein 1; Delta1]

DLL1 (human) (rec.)

AG-40A-0073-C010 — 10 µg
AG-40A-0073-C050 — 50 µg

Produced in HEK 293 cells. Signal peptide and extracellular domain of recombinant human DLL1 (aa 1-545) is fused at the C-terminus to a FLAG®-tag.

DLL1 (human):Fc (human) (rec.)

AG-40A-0116-C010 — 10 µg
AG-40A-0116-C050 — 50 µg

Produced in HEK 293 cells. Signal peptide and extracellular domain of recombinant human DLL1 (aa 1-545) is fused at the C-terminus to the Fc portion of human IgG. Interacts with human Notch1 (as confirmed by flow cytometry).

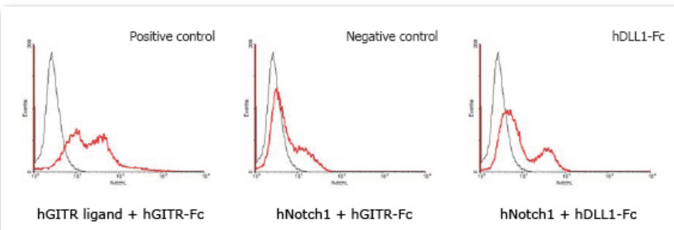

FIGURE: Interaction of human Notch 1 with DLL1 (human):Fc (human) (rec.) (Prod. No. AG-40A-0116).

METHOD: HEK293 cells transfected with a human Notch 1 or a control vector were incubated with 25µg/ml of GITR (human):Fc (human) (rec.) or DLL1 (human):Fc (human) (rec.) (Prod. No. AG-40A-0116). Cells were stained with anti-human IgG (Fc specific) FITC conjugate for DLL1-Fc binding.

new DLL1 (mouse):Fc (human) (rec.)

AG-40A-0148-C010 — 10 µg
AG-40A-0148-C050 — 50 µg

Produced in HEK 293 cells. Signal peptide and extracellular domain of recombinant mouse DLL1 (aa 1-545) is fused at the C-terminus to the Fc portion of human IgG. Interacts with Notch receptors.

anti-DLL1 (human), mAb (D1L165-6)

AG-20A-0074-C050 — 50 µg
AG-20A-0074-C100 — 100 µg

CLONE: D1L165-6. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human DLL1. **SPECIFICITY:** Recognizes human DLL1. **APPLICATION:** WB.

anti-DLL1 (human), pAb

AG-25A-0062-C100 — 100 µg

From rabbit. **IMMUNOGEN:** Recombinant human DLL1. **SPECIFICITY:** Recognizes human DLL1. **APPLICATION:** IHC (PS), WB.

anti-DLL1 (human), pAb

AG-25A-0079-C100 — 100 µg

From rat. **IMMUNOGEN:** Recombinant human DLL1. **SPECIFICITY:** Recognizes human DLL1. **APPLICATION:** WB.

DLL3 [Delta-like Protein 3; Delta3]

DLL3 (human):Fc (human) (rec.)

AG-40A-0113-C010 — 10 µg
AG-40A-0113-C050 — 50 µg

Produced in HEK 293 cells. Signal peptide and extracellular domain of recombinant human DLL3 (aa 1-466) is fused at the C-terminus to the Fc portion of human IgG1.

DLL4 [Delta-like Protein 4; Delta4]

DLL4 (human):Fc (human) (rec.)

AG-40A-0077-C010 — 10 µg
AG-40A-0077-C050 — 50 µg

Produced in HEK 293 cells. Signal peptide and extracellular domain of recombinant human DLL4 (aa 1-529) is fused at the C-terminus to the Fc portion of human IgG. Interacts with human Notch1 (as confirmed by flow cytometry).

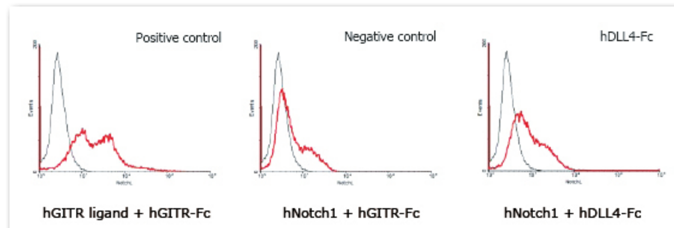

FIGURE: Interaction of human Notch1 with human DLL4 (Prod. No. AG-40A-0077).

METHOD: HEK 293 cells transfected with a human Notch1 or a control vector were incubated with 25µg/ml of GITR (human):Fc (human) (rec.) or DLL4 (human):Fc (human) (rec.) (Prod. No. AG-40A-0077). Cells were stained with anti-human IgG (Fc specific) FITC conjugate for DLL4-Fc binding.

new DLL4 (mouse):Fc (human) (rec.)

AG-40A-0145-C010 — 10 µg

Produced in HEK 293 cells. Signal peptide and extracellular domain of recombinant mouse DLL4 (aa 1-532) is fused at the C-terminus to the Fc portion of human IgG.

new anti-DLL4 (human), mAb (DL86-3AG)

AG-20A-0080-C050 — 50 µg
AG-20A-0080-C100 — 100 µg

CLONE: DL86-3AG. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human DLL4 (ectodomain). **SPECIFICITY:** Recognizes human DLL4. **APPLICATION:** WB.

anti-DLL4 (human), pAb

AG-25A-0080-C100 — 100 µg

From rat. **IMMUNOGEN:** Recombinant human DLL4. **SPECIFICITY:** Recognizes human DLL4. **APPLICATION:** WB.

new DLL1, Soluble (human) ELISA Kit

AG-45A-0027EK-KI01		96 wells
AG-45A-0027TP-KI01	Twin Plex	2 x 96 wells
AG-45A-0027PP-KI01	Penta Plex	5 x 96 wells

For the quantitative determination of soluble DLL1 in human serum, plasma or cell culture supernatant. **SENSITIVITY:** 120pg/ml (range 0 to 16ng/ml).

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation;
IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

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- Notch signaling: the core pathway and its posttranslational regulation: M. E. Fortini; Dev. Cell **16,** 633 (2009)
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- The canonical Notch signaling pathway: unfolding the activation mechanism: R. Kopan & M. X. Ilagan; Cell **137,** 216 (2009)
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- Notch signalling and haematopoietic stem cell formation during embryogenesis: M. Gering & R. Patient; J. Cell Physiol. **222,** 11 (2010)

Dlk1 [Delta-like Protein; Pref-1]

Dlk1 (human) (rec.)

AG-40A-0133-C010 10 µg
AG-40A-0133-C050 50 µg

Produced in HEK 293 cells. The signal peptide and the extracellular domain of human Dlk1 (aa 1-303) is fused at the C-terminus to a FLAG®-tag.

Dlk1 (human):Fc (human) (rec.)

AG-40A-0118-C010 10 µg
AG-40A-0118-C050 50 µg

Produced in HEK 293 cells. The signal peptide and the extracellular domain of human Dlk1 (aa 1-303) is fused at the C-terminus to the Fc portion of human IgG.

Dlk1 (mouse):Fc (human) (rec.)

AG-40A-0107-C010 10 µg
AG-40A-0107-C050 50 µg

Produced in HEK 293 cells. The signal peptide and the extracellular domain of mouse Dlk1 (aa 1-305) is fused at the C-terminus to the Fc portion of human IgG.

anti-Dlk1 (human), mAb (PF13-3)

AG-20A-0069-C050 50 µg
AG-20A-0069-C100 100 µg

CLONE: PF13-3. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human Dlk1 (extracellular domain). **SPECIFICITY:** Recognizes human Dlk1. **APPLICATION:** FC, IHC, WB.

anti-Dlk1 (human), mAb (PF299-1)

AG-20A-0070-C050 50 µg
AG-20A-0070-C100 100 µg

CLONE: PF299-1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human Dlk1 (extracellular domain). **SPECIFICITY:** Recognizes human Dlk1. **APPLICATION:** FC, IHC, WB.

anti-Dlk1 (mouse), mAb (PF105B)

AG-20A-0057-C050 50 µg
AG-20A-0057-C100 100 µg

CLONE: PF105B. **ISOTYPE:** Rat IgG2. **IMMUNOGEN:** Recombinant mouse Dlk1 (extracellular domain). **SPECIFICITY:** Recognizes mouse Dlk1. **APPLICATION:** WB.

anti-Dlk1 (mouse), mAb (PF183E)

AG-20A-0058-C050 50 µg
AG-20A-0058-C100 100 µg

CLONE: PF183E. **ISOTYPE:** Rat IgG2. **IMMUNOGEN:** Recombinant mouse Dlk1 (extracellular domain). **SPECIFICITY:** Recognizes mouse Dlk1. **APPLICATION:** WB.

new anti-Dlk1 (human), pAb

AG-25A-0092-C100 100 µg

From rabbit. **IMMUNOGEN:** Recombinant human Dlk1 (extracellular domain). **SPECIFICITY:** Recognizes human Dlk1. Weakly cross-reacts with mouse Dlk1. **APPLICATION:** WB.

anti-Dlk1 (human), pAb

AG-25A-0091-C100 100 µg

From rat. **IMMUNOGEN:** Recombinant human Dlk1 (extracellular domain). **SPECIFICITY:** Recognizes human Dlk1. **APPLICATION:** FC, WB.

LATEST INSIGHT

Notch Ligands Inhibit Adipocyte Differentiation

Not only Dlk1/Pref-1 [Y. Wang & H.S. Sul; Cell Metab. **9**, 287 (2009)] but also the human Notch ligands DLL1, DLL4, DNER have been shown to inhibit adipocytes differentiation (adipogenesis) [unpublished data], therefore being interesting tools for stem cell research.

Experimental: Adipogenesis Inhibition

3T3L1 cells were maintained in DMEM supplemented with 10% FBS and penicillin-streptomycin. When the cells were confluently grown, adipogenesis was initiated by adding IBMX, dexamethasone, and insulin to 0.5mM, 1µM, and 10µg/ml, respectively and continued for 2 days (day 0). The medium was replaced every 2 days with new medium containing insulin in the presence or absence of 5µg/ml of each human recombinant Notch ligand-Fc fusion protein (human DLL1-Fc, human DNER-Fc, or human Dlk1/Pref-1) and human CD137-Fc as a control-Fc. Staining with Oil Red O was typically performed on day 7.

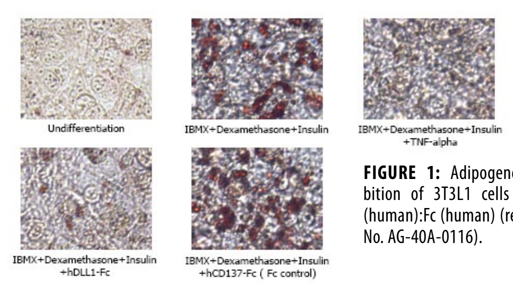

FIGURE 1: Adipogenesis inhibition of 3T3L1 cells by DLL1 (human):Fc (human) (rec.) (Prod. No. AG-40A-0116).

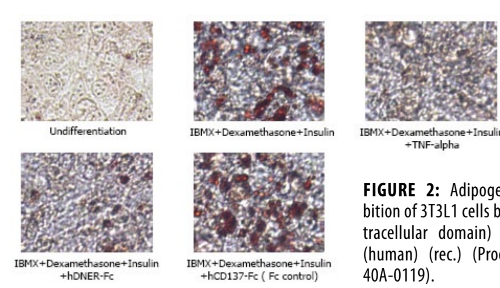

FIGURE 2: Adipogenesis inhibition of 3T3L1 cells by DNER (extracellular domain) (human):Fc (human) (rec.) (Prod. No. AG-40A-0119).

Jagged-1 [HJ1; CD338]

Jagged-1 (human):Fc (human) (rec.)

AG-40A-0081-C010 10 µg
AG-40A-0081-C050 50 µg

Produced in HEK 293 cells. Signal peptide and extracellular domain of human Jagged-1 (HJ1; CD339) (aa 1-1067) are fused at the C-terminus to the Fc portion of human IgG.

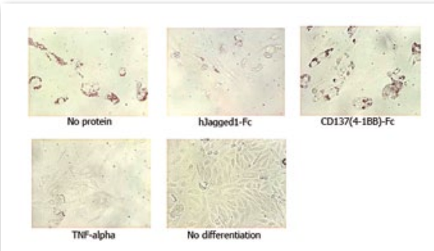

FIGURE: Differentiation of human mesenchymal stem cells (MSCs) into adipocytes in the presence or absence of Notch ligands that inhibit adipogenic differentiation of MSCs.

METHOD: MSCs were maintained in DMEM, supplemented with 10% fetal bovine serum, penicilin-streptomycin and glutamine. For differentiation of MSCs they were cultured in adipogenic medium corresponding to growth medium supplemented with 1µM dexamethasone, 0.5mM IBMX, 10µg/ml insulin and 100µM indomethacin (day 1). Medium was changed every 3 days. Staining with Oil Red O was typically performed on day 30. For negative controls TNF-α (20ng/ml) was added. To immobilize Notch ligands on the plastic surface of the culture plates, plates were incubated with a solution of Jagged-1 (human):Fc (human) (rec.) (Prod. No. AG-40A-0081) (5µg/ml) or CD137L:Fc (5µg/ml) in PBS for 2 hours at 37°C. These plates were then used to differentiate MSCs.

anti-Jagged-1 (human), mAb (J1G74-7)

AG-20A-0050-C100 100 µg

CLONE: J1G74-7. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human Jagged-1. **SPECIFICITY:** Recognizes human Jagged-1. **APPLICATION:** FC, WB.

anti-Jagged-1 (human), pAb

AG-25A-0081-C100 100 µg

From rat. **IMMUNOGEN:** Recombinant human Jagged-1 (extracellular domain). **SPECIFICITY:** Recognizes the extracellular domain and full-length human Jagged-1. **APPLICATION:** WB.

anti-Jagged-1 (human), mAb (J1G53-3)

AG-20A-0049-C100 100 µg
AG-20A-0049F-C050 FITC 50 µg
AG-20A-0049PC-C050 PerCP 50 µg

CLONE: J1G53-3. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human Jagged-1. **SPECIFICITY:** Recognizes human Jagged-1. **APPLICATION:** FC, WB.

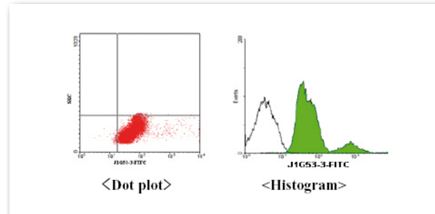

FIGURE: Flow Cytometry. Surface staining method was used to stain normal human peripheral blood cells (CD4+ selections) with anti-Jagged-1 (human), mAb (J1G53-3) (FITC). An appropriate isotype control was used for FITC mouse IgG1.

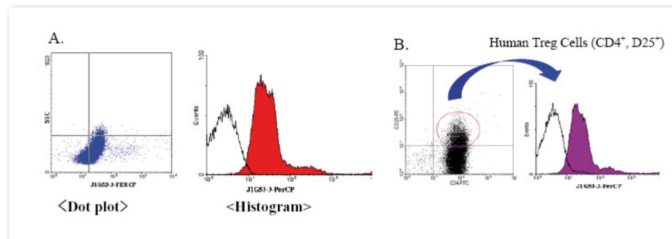

FIGURE: Flow Cytometry.

A: Surface staining method was used to stain normal human peripheral blood cells (CD4+ selections) with anti-Jagged-1 (human), mAb (J1G53-3) (PerCP). An appropriate isotype control was used for PerCP mouse IgG1.

B: Human CD4+ (selected via DYNAL113.03) cells were surface-stained with cocktail of CD4-FITC and CD25-PE subsequently with 20µl/million cells anti-Jagged-1 (human), mAb (J1G53-3) (PerCP) or PerCP mouse IgG1 isotype control using the human regulatory T cell staining set. The dot blot on the left demonstrates co-staining of CD4 and CD25, while the histogram on the right demonstrates J1G35-3 staining of gated CD4+ CD25+ lymphocytes (Regulatory T cells / Treg) versus isotype control. Cells in the lymphocyte gate were used for flow cytometric analysis.

DNER

DNER (extracellular domain) (human) (rec.)

AG-40A-0137-C010 10 µg
AG-40A-0137-C050 50 µg

Produced in HEK 293 cells. The signal peptide and the extracellular domain of human DNER (aa 1-640) are fused at the C-terminus with a FLAG®-tag.

DNER (extracellular domain) (human):Fc (human) (rec.)

AG-40A-0119-C010 10 µg
AG-40A-0119-C050 50 µg

Produced in HEK 293 cells. The signal peptide and the extracellular domain of human DNER (aa 1-637) is fused at the C-terminus to the Fc portion of human IgG.

new anti-DNER (human), mAb (DR324-4)

AG-20A-0078-C050 50 µg
AG-20A-0078-C100 100 µg

CLONE: DR324-4. **ISOTYPE:** Mouse IgG2. **IMMUNOGEN:** Recombinant human DNER (ectodomain). **SPECIFICITY:** Recognizes human DNER. **APPLICATION:** WB.

new anti-DNER (human), pAb

AG-25A-0102-C100 100 µg

From rabbit. **IMMUNOGEN:** Recombinant human DNER (ectodomain). **SPECIFICITY:** Recognizes human DNER. **APPLICATION:** WB.

JAN-2010