Shaping vessels and microenvironment: adipose stromal cells in retinal-related diseases

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Chapter 4

Interplay Between Notch and Jagged is Involved in Adipose tissue-derived Stromal Cells and Endothelial Cells Vascular Assembly

Interplay Between Notch and Jagged Is Involved in Adipose tissue-derived Stromal Cells and Endothelial Cells Vascular Assembly

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Abstract

Cell therapy for the treatment of aberrant vascular network formation caused by diabetic retinopathy warrants detailed understanding of mechanisms of cell-to-cell communication to improve transplantation. Adipose-derived stromal cells (ASC) have a role in promoting and guiding endothelial cells to form and maintain healthy capillaries in the retina. At the same time, to serve as a viable therapeutic option in diabetic retinopathy, transplanted cells need to be resilient to hyperglycemia. Notch signaling is a mechanism that functions upon cell-to-cell contact, directing spatial organization and addressing the cells' identity. In this study; firstly, notch signaling components were analyzed and compared between ASC and endothelial cells in normo- and hyperglycemia conditions. Secondly, levels of notch signaling components were also measured when ASC and endothelial cells were cocultured. Variations in notch components were observed predominantly in cocultured experiments, where NOTCH2 and NOTCH3 had higher expression in hyperglycemic conditions. Interestingly, VEGFA and VEGFR1 were also upregulated in hyperglycemia cocultured cells. Next, inhibition of γ-secretase (notch receptors inhibitor) and JAGGED1 (ligand inhibitor) on a vessel-network formation (VNF) assay showed that notch receptors were important for ASC/endothelial cells communication whereas variations of the ligand levels did not affect VNF in vitro. However, in vivo inhibition of JAGGED1 led to disturbed capillary network development that was partially rescued by ASC. In conclusion, this study showed that ASC promote cell-to-cell communication with endothelial cells through notch receptors whereas notch ligands have a compensatory mechanism for JAGGED1.

Introduction

Hyperglycemia is one of the primary causes of cellular dysfunction in patients affected by diabetes. Excessive glucose uptake dysregulates metabolic pathways and provokes an over production of reactive oxygen species which are toxic for cells [1]. In the eyes, the microcirculation is composed of microvascular endothelial cells that selectively protect the retinal microenvironment from putative dangerous molecules in the bloodstream. Another important component of this system are the pericytes that help to maintain the integrity of the capillaries, which in thus doing form the blood retinal barrier. Diabetic retinopathy is a complication of diabetes that progressively damages endothelial cells and pericytes communication and vessels structures leaving the retinal microenvironment unprotected [2].

Stem cells might be used as surrogates for pericytes; helping to maintain the integrity of the blood retinal barrier. Adipose derived stromal cells (ASC) have many characteristics similar to pericytes. ASC express proangiogenic factors (i.e. smooth muscle actin (SMA), neurogenic factor 2 (NG2), platelet derived growth factor receptor beta (PDGFRB)) and several studies report evidence of the supporting role for providing resilience to oxidative stress. However, the retinal microenvironment includes a multitude of signals which might interfere with ASC differentiation to the pericytic phenotype [3-5].

Notch signaling regulates cells' identity and morphogenesis during development and in pathological conditions. Ligand/receptor interactions lead to activation of target genes in the neighboring cells. We previously demonstrated that NOTCH2 is pivotal for ASC to induce endothelial cells to form vessel like network structures in vitro. Upon ASC NOTCH2 knock down, one of the ASC pericytic markers, PDGFRB, was also downregulated, indicating that ASC had lost their

capacity to function as pericyte-like cells [6]. However, whether notch signaling activation on ASC depends on canonical notch ligands it is not known.

Notch ligands comprise disulphide-rich Delta/Serrate/LAG-2 (DSL) domain with the function of activating in -trans and inhibiting in -cis [7]. Mutation allele in *Jag1* gene was demonstrated to be indispensable for remodeling the embryonic vasculature in mice [8]. Further studies in mice demonstrated that targeting notch pathways at several points such as receptor and ligand (*Notch1* and *Jagged1*) as well as downstream target genes (i.e. *Hey1* and *Hey2*), provoked early embryonic lethality due to vascular defects and deficits in cell fate decisions [9]. Evidence that notch signaling is involved in maintaining stem cells' niches has been demonstrated by Woo and co-workers. In this study, notch signaling was inhibited upstream at the ligand/receptor interaction, and self-renewal and proliferation of neurospheres derived from human embryonic stem cells were reduced [10]. Another study, showed the importance of endothelial JAGGED1 in the communication with stem cells to sustain the formation of blood vessels. Importantly, this study demonstrated that JAGGED1 induced a pericytic phenotype in stem cells isolated from infantile hemangioma [11].

In the context of cell therapy for the stabilization of endothelial cells in diabetic retinopathy, ASC are considered to function as pericytes. In order to improve our understanding of the regulation of ASC pericytic features, we first analyzed the impact of a hyperglycemic environment on the gene expression of notch components in ASC and endothelial cells. Subsequently, the interaction between notch ligands and receptors was inhibited demonstrating that notch signaling is fundamental for ASC pericytic function in terms of endothelial network formation in vitro. Finally, ASC and JAGGED1 inhibitors

were used to investigate the effect on the microvasculature maintenance in vivo.

Methods

Primary cell cultures and isolation

ASC were isolated from lipoaspirates as described previously [12]. Anonymously donated samples were obtained with informed consent as approved by the ethical board of the University Medical Center Groningen following the guidelines for 'waste materials'. Propagation of ASC was in DMEM (BioWhittaker Walkersville, MD): 10% fetal bovine serum (FBS (Thermo Scientific, Helmel Hampstead, UK)), 1% l-Glutamine, 1% Penicillin/Streptomycin (P/S); which was changed for endothelial cells medium (ECM, RPMI-1640 (Lonza Biowhittaker Verviers, Belgium), 10% heat inactivated FBS, 1% P/S, 2 mM L-glutamine (Lonza Biowhittaker Verviers, Belgium), 5U/ml heparin (Leo Pharma, The Netherlands) and 5µg/ml of ECGF growth factor) prior to co-culture experiments. Human umbilical vein endothelial cells (HUVEC) were obtained from Lonza (Breda, The Netherlands) and human microvascular endothelial cells (hmEC, primaty skin-derived cells) from the Endothelial Cell Facility of University Medical Center Groningen, The Netherlands. HUVEC were cultured in ECM and hmEC in DMEM with 5% human serum. ASC and HUVEC were seeded at 4 x $10^4/cm^2$. HUVEC were cultured on gelatin-coated tissue culture polystyrene (TCPS). Immortalized human brain pericytes (HP) (as reported by [13]) were seeded on gelatin coated plates in DMEM. Cells' medium was refreshed every three days. ASC, HUVEC and hmEC were used between passage 3 and 5.

Gene transcript analysis

The gene expression profiles were analyzed in ASC, HUVEC, hmEC and cocultured ASC/hmEC. Total RNA was extracted using Trizol Reagent (Life technologies, Carlsbad, CA) according to the manufacturer's protocol. Afterwards, 1µg of total RNA was reverse transcribed using the First Strand cDNA synthesis kit (Fermentas UAB,

Vilnius, Lithuania) according to the manufacturer's instructions. The cDNA equivalent of 5ng RNA was used for amplification in 384-well microtiter plates in a TaqMAN ABI7900HT cycler (Applied Biosystem, Foster City, CA) in a final reaction volume of 10µl containing 5µl SybrGreen universal PCR Master Mix (BioRad, Richmond, CA) and 6 mM primer mix (forward and reverse). Cycle threshold (C_T) values for individual reactions were determined using ABI Prism-SDS 2.2 data processing software (Applied Biosystem, Foster City, CA). The C_T values were normalized to *GAPDH* as a reference gene using the ΔC_T method, normalizing for the expression of the reference gene and related to the control treatment. All cDNA samples were amplified in duplicate. For primers sequences see chapter 3. (Table 1, page 108)

Capillary-like tube formation assay

µ-Slides for angiogenesis (Ibidi, Munich, Germany) were coated and incubated with growth factor-reduced Matrigel® at 37°C for 30 minutes. ASC, HP and HUVEC were plated at 1 x 10^4 cells for 15 hours. For coculture experiments, ASC/HUVEC and HP/HUVEC were each seeded at 5 x 10^3 cells in each well in RPMI medium. For colocalization staining, ASC were CM-DiI-labeled (Thermo Fisher Scientific, Vybrant® CM-DiI red Molecular Probes, Sanbio, Uden, the Netherlands), whereas HUVEC were Vybrant® CFDA SE labeled (Thermo Fisher Scientific, Vybrant® CFDA SE cell tracer kit-green, Molecular Probes, Sanbio, Uden, the Netherlands). Samples were fixed in 4% paraformaldehyde in PBS for 20min. Images were acquired using a light microscope (Leica Microsystem). Post-processing for imaging was achieved using Angiogenesis Analyzer, ImageJ software [14].

Notch signaling inhibitor

ASC, HP and HUVEC were treated with 5µM of N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S- phenylglycine t-butyl ester (DAPT, Calbiochem No. 565784; Merk KGaA) a γ-secretase inhibitor of notch intracellular domain release following ligands interaction. Similarly, JAGGED1 was inhibited by adding 5µM of DSL Peptide (AnaSpec No. 61298, EGT Corporate Headquarters, Fremont, CA, USA).

Choriolliallantoid membrane (CAM) assay

Fertilized chicken eggs were purchased from Het Anker B.V. (Ochten, the Netherlands) and incubated in an egg incubator (37°C, 60% humidity). Three days after the beginning of incubation, the eggs' tops were perforated in order to create an air chamber. Four days later, the holes on the eggs' tops were enlarged and a solution of PBS, ASC (1 x 10^4 cells) and/or JAGGED1 inhibitor were added on the capillaries of the CAM. The eggs were left undisturbed for three additional days. Subsequently, CAM were excised for images acqusition by fluorescence stereomicroscope (Leica M10). Capillaries density was measured and analyzed by Angiogenesis Analyzer.

Statistical analysis

All the data were analyzed using GraphPad Prism 5 (GraphPad Software Inc.). Multiple group comparisons were determined using either one-way ANOVA with Bonferroni post-hoc analysis or two-way ANOVA. Data are plotted as averaged values from independent experiments ± standard deviation (SD). Values of $p<0.05$ were considered statistically significant.

Results

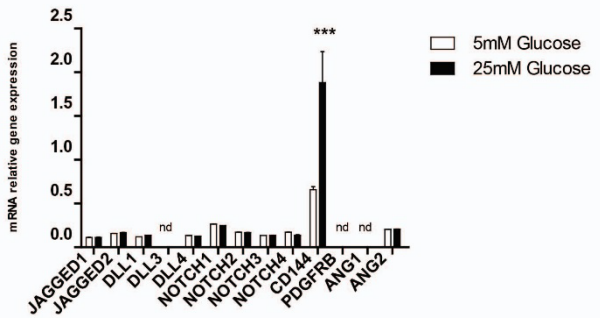
Notch components gene expression variation in normoglycemia and hyperglycemia treated ASC and endothelial cells

In this experiment notch ligands and receptors gene expression were measured in normoglycemia (5mM of glucose) and hyperglycemia (25mM of glucose) conditions. In addition, selected genes involved in angiogenesis and vascular homeostasis were considered. In HUVEC, JAGGED1, JAGGED2, DLL1, DLL4, NOTCH2, NOTCH3 and NOTCH4 had equal expression levels in both normo- and hyperglycemia (Ct value ~0.150 normalized to GAPDH) (Fig. 1). NOTCH1 had higher expression (0.263±0.005), while DLL3 was not detectable. CD144 (Cadherin 5) was found to have higher expression in hyperglycemia (1.883±0.5) compared to normoglycemia (0.656±0.052). ANG2 had similar expression in both conditions (0.2±0.004), while PDGFRB and ANG1 were not detectable in HUVEC (Fig. 1).

Subsequently, the gene expression levels of notch components were compared across three different groups: ASC, hmEC and VNF (coculture of hmEC and ASC) (Fig. 1B). Cells were cultured in normo- and hyperglycemia conditions. There was no variation in the expression of NOTCH1, JAGGED1 and genes involved in vascular maintenance such as ANG1 and ANG2 between the different cell groups. NOTCH2 had similar expression in ASC (NG 0.411±0.018, HG 0.469±0.040), hyperglycemia cultured hmEC (0.396±0.078) and VNF (0.419±0.153). In contrast, normoglycemia treated hmEC (0.188±0.021) and VNF (0.271±0.012) had significantly lower expression compared to hyperglycemia treated cells. NOTCH3 on the other hand, was found to have higher expression when ASC and hmEC (VNF) were cultured together in hyperglycemic conditions (0.252±0.056).

The analysis of notch gene expression components in hmEC and VNF (ASC + hmEC), with the addition of genes involved in angiogenesis is displayed in figure 2A. NOTCH2 (NG 0.366 ± 0.001, HG 0.287 ±0.029) and NOTCH3 (NG 0.228 ±0.006, HG 0.200 ±0.016) genes were confirmed to increase when ASC and hmEC were cocultured compared to hmEC alone (NOTCH2 ~0.2, NOTCH3 ~0.1). The other genes measured; JAGGED1, JAGGED2, DLL1, DLL3, DLL4, ANG1, ANG2 and HEY1 had similar expression in all the conditions and cells. Notably, NOTCH1 and VEGFR2 had one Ct value higher compared to the latter genes listed. However, differences between normo- and hyperglycemia were not detected. Lastly, differences between cultured hmEC and VNF were detected with regard to expression of VEGFA and VEGFR1. In hmEC the VEGFA level in normoglycemia and hyperglycemia was 0.060 ±0.006 and 0.066± 0.004, while in VNF the level was 0.116 ±0.014 and 0.122 ±0.018. VEGFR1 level in hmEC treated in normoglycemia and hyperglycemia was 0.129 ±0.028 and 0.098± 0.016 respectively, while in VNF these levels were 0.238 ±0.001 and 0.179 ±0.034 respectively.

A



B

		NOTCH1	NOTCH2	NOTCH3	JAGGED1	ANG1	ANG2
ASC NG	**Mean**	0.157	0.411	0.138	0.152	0.114	0.097
	SD	0.004	0.018	0.005	0.005	0.004	0.007
	Stat	ns	***hmEC NG	ns	ns	ns	ns
ASC HG	**Mean**	0.162	0.469	0.139	0.138	0.128	0.085
	SD	0.004	0.040	0.008	0.013	0.001	0.005
	Stat	ns	***VNF NG	*VNF HG	ns	*VNF HG	ns
hmEC NG	**Mean**	0.197	0.189	0.110	0.099	0.101	0.095
	SD	0.008	0.021	0.010	0.001	0.007	0.004
	Stat	ns	***hmEC HG	ns	ns	ns	ns
hmEC HG	**Mean**	0.192	0.396	0.176	0.118	0.119	0.109
	SD	0.002	0.078	0.025	0.009	0.002	0.002
	Stat	ns	**VNF NG	ns	ns	ns	ns
VNF NG	**Mean**	0.234	0.272	0.195	0.110	0.129	0.107
	SD	0.006	0.012	0.011	0.004	0.011	0.006
	Stat	ns	**ASC NG/VNF HG	**VNF HG	ns	ns	ns
VNF HG	**Mean**	0.197	0.420	0.253	0.126	0.137	0.117
	SD	0.011	0.153	0.056	0.013	0.005	0.004
	Stat	ns	***hmEC NG	*ASC NG	ns	ns	ns

Figure 1. NOTCH2 and NOTCH3 are enhanced by hyperglycemia in hmEC and VNF. Cells were cultured in monolayer in normoglycemia (5 mM) and hyperglycemia (25 mM) and harvested at confluency before total RNA was isolated. Notch components and angiogenic genes relative gene expression in (A) HUVEC, (B) ASC, hmEC and VNF (ASC/hmEC) was determined by RT-qPCR. Heatmap shows highly expressed genes (red) and lower gene expression (blue). Significant difference between groups are shown beneath single group values. Gene expression was normalized to the reference gene GAPDH (n=2). *, $p \leq 0.05$, ***, $p \leq 0.001$, two-way ANOVA. Nd: not detectable.

	hmEC NG			hmEC HG			VNF NG			VNF HG		
	Mean	SD	Stat	Mean	SD	Stat	Mean	SD	Stat	Mean	SD	Stat
JAGGED2	0.106	0.007	*hmEC HG	0.073	0.001	***VNF NG	0.124	0.011	***VNF HG	0.070	0.015	*hmEC NG
DLL1	0.080	0.002		0.077	0.001		0.081	0.001		0.079	0.002	
DLL3	0.065	0.006		0.067	0.006	**VNF NG	0.105	0.008		0.091	0.008	
DLL4	0.104	0.002		0.109	0.001		0.103	0.001		0.085	0.004	
NOTCH4	0.097	0.004		0.089	0.001		0.110	0.002		0.091	0.006	
HEY1	0.081	0.001		0.080	0.000		0.082	0.001		0.093	0.001	
VEGFA	0.061	0.006	***VNF NG	0.066	0.004	***VNF NG/HG	0.116	0.014		0.122	0.018	***hmEC NG
VEGFR1	0.129	0.028	***VNF NG	0.099	0.016	***VNF NG/HG	0.238	0.001	***VNF HG	0.179	0.034	***hmEC NG
VEGFR2	0.153	0.029		0.147	0.006		0.177	0.004		0.154	0.011	

Figure 2. Coculturing ASC and hmEC enhanced VEGFA and VEGFR1 gene expression. Cells were cultured in monolayer in normoglycemia (5 mM) and hyperglycemia (25 mM) and harvested at confluency, before total RNA was isolated. Notch components and angiogenic genes relative gene expression of VNF (ASC/hmEC) was determined by RT-qPCR. Heatmap shows highly expressed genes (red) and lower gene expression (blue). Significant difference between groups are shown beneath single group values. Gene expression was normalized to the reference gene GAPDH (n=2). ***, $p \leq 0.001$, two-way ANOVA.

γ-secretase and JAGGED1 interfere with vascular density and cells' communication between ASC and endothelial cells

Notch signaling is involved in the development of nascent vessels. On the one hand, receptors and ligands lead endothelial cells to form orderly capillaries. On the other hand, pericytes differentiate toward a plastic phenotype which confers the characteristic of a supportive cell. In order to show that notch signaling plays a role in the communication and network formation between ASC and endothelial cells, DAPT, a γ-secretase inhibitor was used in a capillary-like tube formation assay (Fig. 3). ASC and HUVEC formed an interconnected network which was followed for 12 and 15 hours. When DAPT was added to the coculture, the networks lost continuity and connections (Fig. 3A and C). After 12h DAPT coculture treatment, the communication of ASC (green) and HUVEC (red) remained intact (Fig. 3B). At 15 hours, some of the ASC lost contact with the endothelial network when DAPT was present in the coculture. Similarly, human pericytes (HP) cocultured with HUVEC followed the same pattern, that is endothelial network formation was decreased when DAPT was added to the coculture system (Fig. 3E and G). In contrast, a majority of HP (green) were found not participating and rather scattered when DAPT was present in the coculture with HUVEC (red) (Fig. 3F and H)

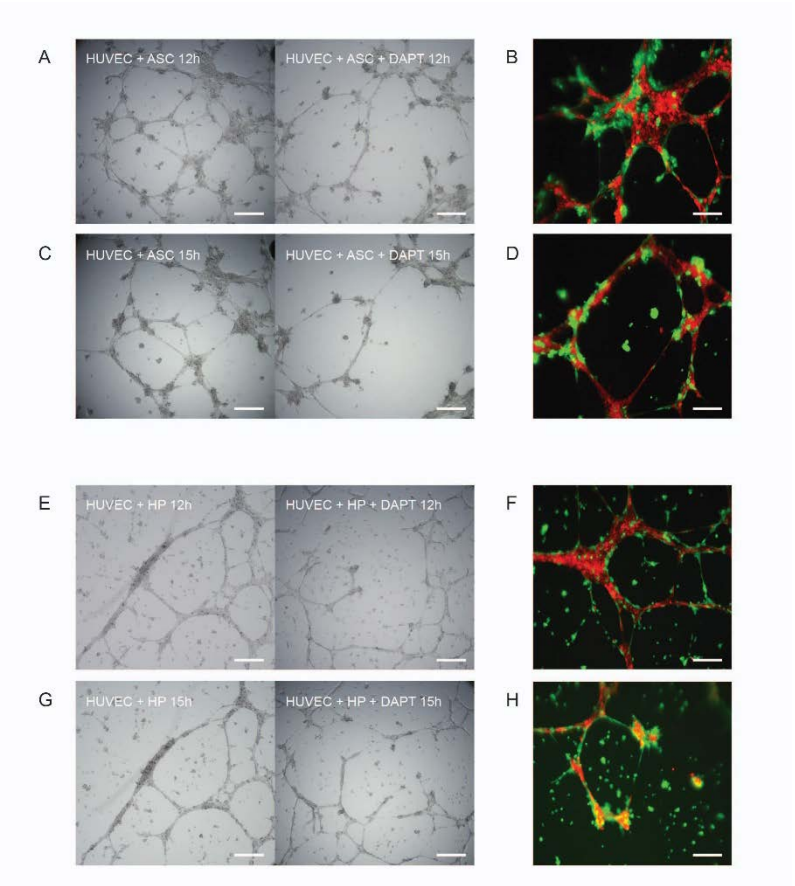


Figure 3. γ-secretase inhibitor reduced vessel network formation. Combination of HUVEC/ASC and HUVEC/HP were seeded on matrigel for 12h and 15h. (A) HUVEC/ASC vessel network formation 12h (control, right), +DAPT (left). (B) VNF of ASC in pericytic position (green) and HUVEC formed tube-like network (red). (C) HUVEC/ASC vessel network formation 15h (control, right), +DAPT (left). (D) VNF+DAPT of ASC in pericytic position (green) and HUVEC formed tube-like network (red). (E) HUVEC/HP vessel network formation 12h (control, right), +DAPT (left). (F) VNF of HP in pericytic position (green) and HUVEC formed tube-like network (red). (G) HUVEC/HP vessel network formation 15h (control, right), +DAPT (left). (H) VNF+DAPT of HP in pericytic position (green) and HUVEC formed tube-like network (red). Scale bar (A,C,E,G) 100µm, (B,D,F,H) 50 µm.

To specifically block the activation of notch signaling through one of its receptors, a JAGGED1 inhibitor was employed. Singularly cultured populations of HUVEC, ASC and HP sprouted on a layer of matrigel (Fig. 4A). Subsequently, the JAGGED1 inhibitor was added. HUVEC maintained the network as the control, whereas ASC and HP showed reduced capacity to form interconnected structures in the presence of the inhibitor (Fig. 4B). Next, the capacity of JAGGED1 to participate in a capillary-like tube network formation was tested in a coculture system with ASC/HUVEC and HP/HUVEC (Fig. 4C). Interestingly, both networks of ASC/HUVEC and HP/HUVEC treated with JAGGED1 inhibitor did not differ from their respective controls in terms of time of sprouting and interconnected structures (Fig. 4D).

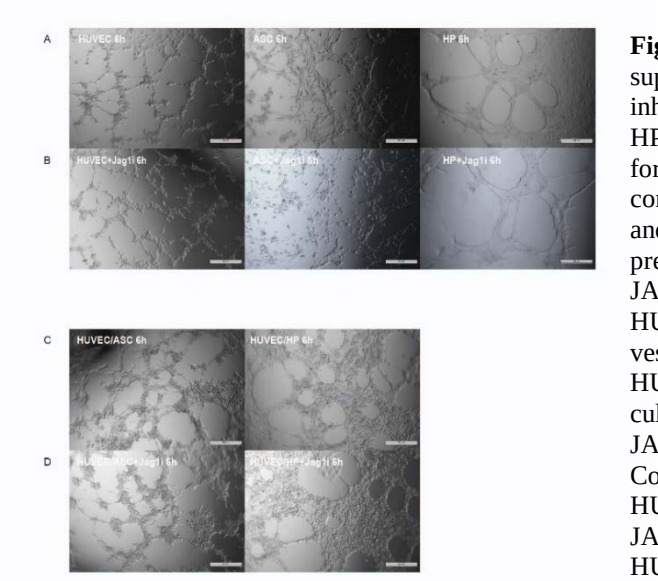


Figure 4. VNF is not suppressed by JAGGED1 inhibitor. HUVEC, ASC and HP were cultured on matrigel for sprouting assays as well as combination of HUVEC/ASC and HUVEC/HP, in the presence or absence of a JAGGED1 inhibitor. (A) HUVEC, ASC and HP formed vessel-like networks. (B) HUVEC, ASC and HP cultured in the presence of JAGGED1 inhibitor. (C) Cocultured HUVEC/ASC and HUVEC/HP VNF. (D) JAGGED1 inhibitor treated HUVEC/ASC and HUVEC/HP. Scale bar 200µm.

In vivo capillaries treated with JAGGED1 inhibitor are rescued by ASC

We previously showed that JAGGED1 inhibitor did not interfere with the capacity of either HUVEC or the combination of ASC/HUVEC to undergo capillary-like tube formation on a layer of matrigel. In order to extend this observation in an in vivo model, the capillaries in development from a choriolliallantoid membrane (CAM) were used. ASC were placed on a CAM and not visible differences were observed compared to the control. The presence of the JAGGED1 inhibitor on the other hand, reduced the formation of capillaries. In the last condition, ASC were added to the capillaries concomitantly treated with JAGGED1 inhibitor. In this case, a recovery in capillaries formation was observed (Fig. 5A). The capillaries networks were measured as the number of honey combs formed (Fig. 5B). From the measurement emerged an increase in capillaries formation in the CAM treated with ASC. The JAGGED1 inhibitor treated CAM had a reduced amount of capillaries formation. The number of replicates of this experiment was too small for statistical analysis. Further replicates are needed to validate these findings.

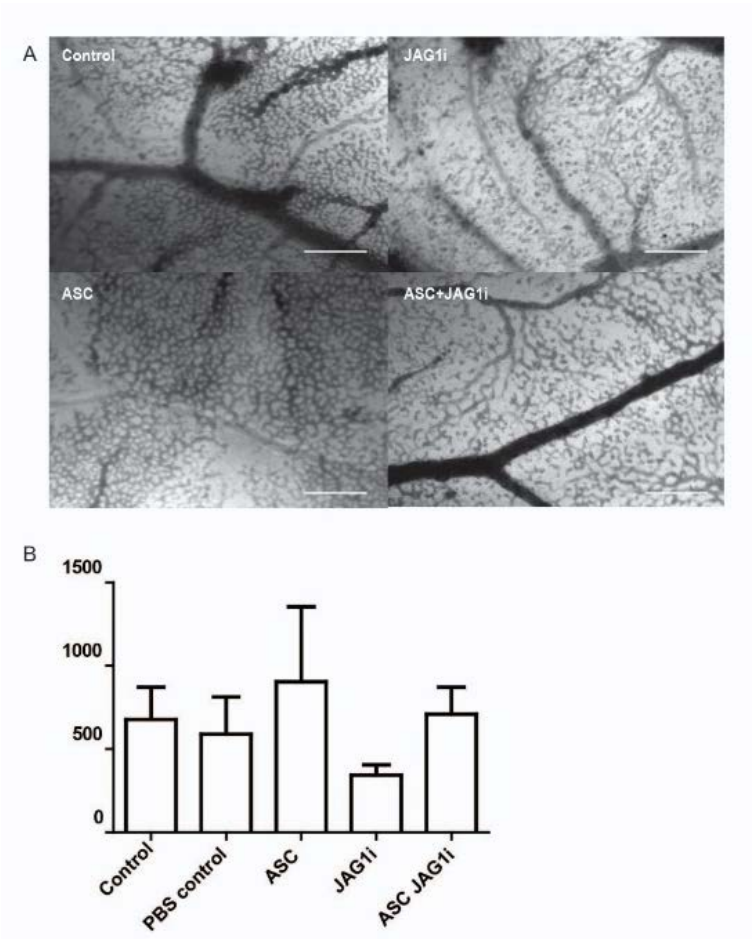


Figure 5. ASC partially rescued JAGGED1 inhibition induced capillaries retraction. Choriolliallantoid membrane (CAM) was used as an in vivo model to test capillaries development under the action of JAGGED1 inhibitor and ASC. (A) Upper left panel shows untreated CAM. Upper right panel shows CAM treated with JAGGED1 inhibitor. Bottom left panel shows ASC added on to capillaries of developmental CAM. Bottom right panel displays combination of ASC and JAGGED1 inhibitor on CAM. (B) Quantification of capillaries in CAM based on number of honey combs formed (n=3). Scale bar: 100µm

Discussion

The effect of hyperglycemia on the notch components of ASC and endothelial cells was reported. We found that at gene levels, notch ligands and receptors remained at a similar level in all the cell populations analyzed, with the exception of NOTCH3 which showed increased mRNA levels when ASC and hmEC were cultured together in high glucose. Moreover, the message of proteins involved in the angiogenic process showed increased expression of VEGFA and VEGFR1. DAPT, an γ-secretase inhibitor, disturbed in vitro capillary-like tube formation of ASC and HUVEC. A JAGGED1 inhibitor had no effect on the ASC/HUVEC coculture. In contrast, in an in vivo model of capillaries formation, JAGGED1 inhibitor disturbed the formation of blood vessels which was partially rescued by ASC.

Possible mechanisms that modulate sprouting angiogenesis include the cross-talk between notch signaling and VEGF signaling. In context, VEGFA binds to VEGFR2 and promotes DLL4 expression in tip cells. DLL4 activates NOTCH1 in neighboring cells which acts as a putative transcriptional repressor of VEGFR2, therefore promoting stalk cells expression of VEGFR1, that in turn becomes less responsive to pro-angiogenic VEGFA (reviewed in [15]). In line with these findings, we showed that expression levels of VEGFR1 were higher in cocultured ASC and endothelial cells. Strikingly, both pro-angiogenic expression of VEGFA and VEGFR2 were lower compared to VEGFR1. Notably, ASC expression of endogenous VEGFA was described to play an insignificant role in the context of ASC regenerative potential [16]. In addition, notch pathway comprises JAGGED1 which in the developmental retinal vasculature is found to be expressed at endothelial cells branching points and in mural cells/pericytes [17]. JAGGED1 has a bi-directional mode of action that signals with both notch receptors on adjacent cells and on the cells where it is expressed [18]. In the vasculature, JAGGED1 participates in the stability and

interaction of pericytes to promote a vascular smooth muscle cell phenotype [19]. We found no alteration by hyperglycemia on ASC expressing JAGGED1. JAGGED1 inhibitor however, dysregulated communication of ASC when cultured on matrigel for tube-like formation assay. In contrast, JAGGED1 inhibitor did not influence the tube formation of cocultured ASC and endothelial cells. JAGGED1 has been shown to interact with NOTCH1, NOTCH2 and NOTCH3. This interaction is modified by a glucosyltransferase Fringe which might explain the differential affinity of Serrate and Delta [20,21]. In fact, in a previous study we showed that ASC NOTCH2 expression was indispensable for promoting communication and subsequent vessel formation by endothelial cells in vitro [6]. Since notch ligand JAGGED1 on ASC did not influence capillary-like tube formation in vitro, most likely other ligands on endothelial cells bind to ASC notch receptors. Further research is needed to explore these ligands in the future.

Urs and co-workers demonstrated that soluble JAGGED1 increased angiogenesis in preadipocytes and endothelial cells. At the same time, notch receptors were downregulated [22]. These data hint to a pro-angiogenic role of JAGGED1 expressing ASC. Therefore, we asked the question whether ASC would rescue aberrant microvasculature caused by JAGGED1 inhibition in vivo. In diabetic mice, hyperglycemia induces overexpression of JAGGED1, resulting in endothelial cells developing into an uncontrolled angiogenic sprout. In the same study, pathological angiogenesis in the retinal microcirculation was reduced upon JAGGED1 down regulation [23]. In our model, chorioallantoic membrane was treated with JAGGED1 inhibitor and regression of capillaries formation was observed. ASC partially rescued this phenotype by increasing the number of capillaries.

In conclusion, we demonstrated that ASC can modulate angiogenesis and vascular sprouting through a mechanism involving notch signaling. On the one hand notch receptors are indispensable for ASC communication with endothelial cells. On the other hand, JAGGED1 does not influence communication, suggesting other ligands might counterbalance the activity of notch receptors on ASC. In the context of cell therapy in the eyes, ASC have the potential to function as pericytes through notch signaling by promoting homeostasis and, depending on the stage of retinopathy, reduce pathological vasoproliferation. JAGGED1 is a promising novel therapeutic target because it is upregulated in pathological conditions contributing to uncontrolled angiogenesis. Engineering ASC that lack JAGGED1 could regulate retinal angiogenic sprouting returning the balance toward homeostasis.

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Part II

Extracellular matrix remodeling

New approaches to study vasculogenesis in vitro