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Notch-1 decreased expression contributes to leptin receptor downregulation in nasal epithelium from allergic turbinates

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ABSTRACT

Background: Allergic rhinitis is characterized by a remodeling of nasal epithelium. Since the Notch and TGF-β signaling pathways are known to be involved in cell differentiation and remodeling processes and leptin adipokine has already been identified as a marker for homeostasis in human bronchial and nasal epithelial cells of asthmatics, roles played by these pathways have been investigated for chronic allergic rhinitis.

Methods: The leptin/leptin receptor expression has been investigated in a study with 40 biopsies from allergic (AR, n = 18) and non-allergic (C, n = 22) inferior turbinates, using immunohistochemistry, immunofluorescence staining and RT-PCR. In addition, extracts from *in vitro* samples prepared from primary cells of inferior turbinates as well as *in vitro* cultured human nasal epithelial RPMI 2650 cells (ATCC-CCL-30) were also tested for leptin expression and activation of the Notch-1 pathway.

Results: With regards to AR, *in vivo* expression levels of both leptin and its receptor significantly decreased in comparison to C. Furthermore, leptin receptor mRNA was significantly reduced in AR as compared to C. Immunofluorescence showed an apparent co-expression of leptin receptor with Notch-1, which was not seen with TGF-β. *In vitro*, in primary turbinate epithelial cells, the expression of leptin receptor and Notch-1 significantly decreased in AR as compared to C. Moreover, in RPMI 2650 cells, leptin receptor expression was shown to be induced by Notch-1 ligand signaling.

Conclusion: Thus, both the leptin and Notch-1 pathways appear to represent markers for epithelial homeostasis in allergic rhinitis.

1. Introduction

Allergic rhinitis is a global health problem and usually persists throughout life. The prevalence of allergic rhinitis confirmed in adults in Europe ranges from 17% to 28.5% [1]. Although allergic rhinitis is not associated with severe morbidity or mortality, it broadly affects the quality of life of patients owing to the frequent symptoms such as nasal congestion, sneezing and rhinorrhea which lead to irritability, fatigue and increased costs for the medical care. Allergic airway diseases can range from the nose to lungs as the upper and lower respiratory tracts share many histologic, functional and immunological features [1,2]. Many studies have demonstrated parallel increases in the prevalence of asthma and rhinitis and the new update of *Allergic Rhinitis and its Impact on Asthma* (ARIA) concludes that allergic rhinitis is one of the multiple risk factors for asthma development and proposes that combined strategy should be developed for treating the upper and lower airways for a better efficacy/safety ratio [3,4].

In the meantime, several reports indicate that leptin, a pleiotropic adipokine involved in immune system regulation, maintains and preserves bronchial and nasal epithelial homeostasis in epithelium affected by asthma and allergy. In contrast, there is a decreased expression of the pro-fibrogenic TGF-β activity in inflamed epithelium in comparison to non-damaged epithelium [5,6]. Particularly in nasal epithelial cells, human recombinant TGF-β1 is able to significantly decrease cell proliferation, without significantly decreasing leptin receptor expression either as mRNA or as protein [6]. With the turbinates, previous studies performed in animals report that leptin increases the production of mucus in olfactory globular cells, thus suggesting that leptin might be a potential target for physiological modulation of mucus composition and for regulation of olfactory functions such as neuromodulation or

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cellular homeostasis [7,8]. Leptin signaling in the respiratory tract is known to regulate cell survival and proliferation *via* the NF-κB-STAT3 pathway [6,9], as well as with Notch expression that it is associated to angiogenesis, proliferation and differentiation [10].

The Notch family consists of four receptors (Notch1-Notch4) and five ligands: Jagged (JAG1, JAG2) Delta-like (DLL1, DLL3, and DLL4). Loss of regulation of Notch signaling are critically linked to the pathogenesis of various lung diseases, included asthmatic airway remodeling [10]. Notch signaling can interact with other signaling such as NF-κB and TGF-β [11]. Particularly, in a process known as epithelial-mesenchymal transition (EMT), in lung cancer the crosstalk between Notch and TGF-β is imperative for induction of EMT because Notch signaling is required to maintain TGF-β-induced expression [10]. In breast cancer, Notch can be induced and activated by leptin signaling [12,13]. Furthermore, the Notch signaling pathway is increased in nasal epithelium with differentiation abnormalities in patients suffering from nasal polyposis and it contributes to pro-inflammatory responses [14]. Simultaneously, previous evidence indicates a significant increase of leptin receptor expression in nasal polyps as compared to normal nasal mucosa, thus suggesting that leptin may play a role in inflammation in nasal polyposis [15]. At the moment, no data have been reported on the relationship between leptin receptor pathway and Notch signaling in human epithelium from the turbinates of subjects affected by allergic rhinitis. The present study was performed to establish the role of the leptin/leptin receptor pathway in the epithelial homeostasis in allergic rhinitis and to investigate whether deregulation of Notch-1 signaling might contribute to alterations in the leptin/leptin receptor pathway in chronic allergic rhinitis as already demonstrated in bronchial asthma.

2. Materials and methods

2.1. Reagents

The polyclonal anti-leptin receptor antibody (Ob-R M-18, sc-1834), against the common part of the short and long forms of leptin receptor, and the anti-leptin antibody (Ob A-20, sc-842), the DAPT peptide blocking Notch-1 signaling (LY-374973, sc-201,315), were from Santa Cruz Biotechnology. The monoclonal anti-TGF-β1,2,3 antibody (MAB1835) and the active fragment of human Jag-1 protein (aa 188–204) (188–204, AS-61298, AnaSpec, San Jose, CA, USA) which activates Notch-1 signaling (Jagged-1), were purchased from R&D Systems and Eurogentec, respectively. The primary monoclonal Notch-1 (A6, MA5–11961) and CD326 (EpCAM, eBioscience™ 1B7, 14–9326-82) antibodies were obtained from Invitrogen. Polyclonal Rabbit Anti-Goat Immunoglobulins/FITC for leptin receptor (F 0250), the REAL™ Detection System, AlkalinePhosphatase/RED, Rabbit/Mouse (K5005), the Polyclonal Rabbit Anti-Goat Immunoglobulins/Biotinylated (E0466), the conjugated secondary antibodies FITC (F0250) and PE (R0439) were from Dako. Conjugated secondary antibodies Alexa Fluor 488–555 were purchased from Invitrogen. Normal donkey (NDS, 017–000-121) and Normal goat serum (NGS, 005–000-121) were from Jackson Immunoresearch. The RNeasy FFPE Kit (n 73,504, Qiagen), Superscript first-strand synthesis system for RT-PCR (n 11,904–018, Invitrogen), FAM-labeled probe and primers (Applied Biosystems) were employed.

2.2. Study design and subjects enrolled

All subjects recruited (n 40; 39.6 years; M = 75%) with nasal obstruction (100%) were with allergic rhinitis (AR, n = 18) or non-allergic rhinitis matched subjects as controls (C, n = 22), selected at the ENT Polyclinic, University of Palermo, a tertiary care referral center in Palermo, Italy. All patients were recruited according to the ARIA criteria [1]. The atopic status was established by positivity to one or more of routinely tested aeroallergens by skin prick test (SPT). SPT was performed with mixed grass pollens (lolium perenne, phleum pratense, cynodon dactylon), Olives, Mugworth, Parietaria Judaica, Mites, Alternaria, Cat, Dog, and Peach were performed with all study participants using standardized extracts (Alk Abellò, Hørsholm, Denmark). All patients discontinued therapy for rhinitis one month before surgery and underwent nasal surgery for chronic sinusitis or deviation of the nasal septum. In all patients a volumetric reduction of the inferior turbinates with radiofrequency was performed. During this phase of surgery, a mucosa sampling of the inferior turbinate was carried out. This procedure was performed with cold instruments to avoid damaging the mucosal structure. Particularly, from inferior turbinates of selected patients (C, n = 4; AR, n = 5), total cells were extracted and stained for primary epithelial cells (Epcam +), followed by flow cytometry analysis. The study protocol was approved by the Polyclinic Ethics Committee (13/2013) of Palermo and informed written consent was obtained from each patient. The study was carried out in agreement with the Helsinki Declaration.

2.3. Immunohistochemistry and immunofluorescence

Inferior turbinates biopsies were fixed in 10% formaldehyde (pH 7.2) and embedded in paraffin. Five micrometer tissue sections were attached to polylysine-coated microscope slides, and, after dewaxing and rehydration, were stained with hematoxylin or analyzed with immunohistochemistry or immunofluorescence. Immunohistochemistry was performed at RT for leptin (1:40), at 4 °C overnight for leptin receptor (1:50) and for TGF-β1,2,3 (25 μg/ml). The immunoreactions were detected using the Dako REAL™ Detection System. In the epithelium, positive cells of each studied marker were evaluated blindly by two independent investigators (AB and GC), per area over a minimum length of 260 mm from the internal side of the epithelium [5] using a Quantimet 500 MC software (Leica) for image analysis. Immunofluorescence was performed on tissue sections after treatment for antigen retrieval with 10 mM citrate buffer (pH 6.0). The sections were blocked with PBS plus 10% NDS/NGS and immunofluorescent stainings were performed at room temperature for 2 h using the following primary Abs diluted in PBS plus 2% NDS/NGS: anti-leptin, leptin receptor, TGF-β1,2,3 (at the same concentration as described above) and Notch-1 (1:80). Incubation with appropriate Alexa Fluor 488–555 secondary antibodies (1:500) was performed for 30′. Double immunofluorescent staining was also performed for leptin/leptin receptor and TGF-β1,2,3 and for leptin receptor/Notch-1. Sections on coverslips were mounted in Vectashield (Vector Laboratories, Burlingame, CA) with 406-diamidine-2-phenylindole-dihydrochloride (DAPI) to visualize nuclei. Staining of the samples was observed and evaluated with an Axioskop-2-Zeiss or LEICA DM 4000 microscopes/software. Control slides were prepared using an irrelevant antibody of the same isotype and at the same concentration as the specific primary and no significant staining or fluorescence signals was detected (data not shown).

2.4. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) for leptin and leptin receptor

To purify total RNA from inferior turbinate the RNeasy FFPE Kit was used following the procedure recommended by the manufacturer. Four sections of 10 μm were cut for representative samples (healthy controls, n = 3; rhinitis allergics, n = 3) and RT-PCR was performed by used the superscript first-strand synthesis system as indicated in procedures. For each sample, 300 ng of total extracted cellular RNA was reverse-transcribed. Quantitative real-time PCR was performed by TaqMan Assay. Quantitative real-time PCR for leptin was performed, but could not be detected (data not shown). Quantitative real-time PCR for the common part of the short and long form of human leptin receptor transcripts was carried out with Step One Plus Real-time PCR System (Applied Biosystems, Foster City, CA, USA) using specific FAM-labeled probe and primers (prevalidated TaqMan Gene expression assay for leptin

receptor, Hs00174492m1; Applied Biosystems). Leptin receptor gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (prevalidated TaqMan Gene expression assay for GAPDH, Hs03929097g1; Applied Biosystems) endogenous control gene [16].

2.5. Cell cultures and flow-cytometry

Primary nasal epithelial cells were freshly obtained from surgical specimens of the inferior turbinate (healthy controls, n = 4; rhinitis allergics, n = 5) as previously described [17] and cultured in complete culture medium (MEM minimum essential media containing 10% FCS, L-glutamine 2 mM, gentamicin 50 mg/ml, MEM non-essential amino acid (NEAA) 0.5%, sodium pyruvate 1 nM, HEPES 1% supplied by Gibco, BRL, Germany). Fresh cells were labeled in the dark at RT for 1 h for EpCAM (0.25 μg/100 μl), leptin receptor (1:25) and Notch-1 (1:100), alone or combined. RPMI 2650 cell lines (ATCC-CCL-30) purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA) were also cultured as described above in the presence or absence of Jagged-1 (5 μg/ml) alone or combined with DAPT (0.75 μg/ml) for 24 h for assessing leptin receptor expression. All cell staining was performed after fixing with PBS containing 4% paraformaldehyde, followed by washing twice in permeabilization buffer (PBS containing 1% FBS, 0.3% saponin, and 0.1% Na azide) and incubation with appropriate PE- and FITC-conjugated secondary antibodies. Marker expression was evaluated using a FACS-Calibur (Becton–Dickinson, Mountain View, CA, USA) flow cytometer. Nonimmune IgGs at the same concentration as the primary antibody was used as negative controls. Percentages of positive cells were determined from forward and sideways scatter patterns. Nonspecific binding and background fluorescence were quantified by analyzing the negative controls.

2.6. Statistical analysis

In vivo samples were analyzed with a non-parametric Mann Whitney *U* test applied between the two groups. Correlations were determined with a Spearman rank correlation test. *In vitro*, the analysis of results was performed with at least 4 independent biological replicates (described in figure legends). Analysis of variance (ANOVA) was used for testing differences between means. The possible association between categorical variables was evaluated by the accurate *Fisher's PLSD* exact test (P-values were obtained for log transformed data). If no data from individual experiments are presented, then the figures show mean levels ± SD. A P value < .05 was considered statistically significant.

3. Results

3.1. Characteristics of the subjects

The demographic characteristics, pulmonary functions and medians and 25th–75th percentiles of leptin, leptin receptor and TGF-β1,2,3 expression in the two groups studied are reported in Table 1.

3.2. Leptin/leptin receptor pathway versus TGF-β expression in turbinate epithelium

The expression of leptin and leptin receptor in the epithelium of inferior turbinates from C and AR is assessed here for the first time. The expression of both leptin receptor and leptin is significantly lower in AR than in C (P = .003 and P = .006, respectively), whereas the expression of TGF-β1,2,3 is unaltered and does not co-localize with leptin/leptin receptor expression (Table 1, Figs. 1–2, Fig. S1). Furthermore, a positive correlation is found between leptin receptor and leptin expression (P = .019; Rho = 0.38) (Figs. 3 and 4, A). We did not find any correlation among leptin, leptin receptor and BMI (data not shown). In agreement, the mRNA levels for leptin receptor were significantly lower in AR than in C (P = .04) (Fig. 4, B).

Table 1
Demographic characteristics of the subjects and the epithelial markers.

	Non-allergic rhinitis subjects (C)	Allergic rhinitis patients (AR)	P values
n	22	18	
Age (y)	43.00 (29.25–47.00)	39.50 (23.00–49.00)	0.63
Sex (M/F ratio)	87%	62%	0.1
BMI (kg/m2)	24.91 (22.06–27.49)	25.00 (23.76–31.68)	0.24
FEV1 (% predicted)	94 (87.70–106.25)	98.00 (88.50–106.25)	0.7
FVC (% predicted)	98 (86.20–103.25)	103.5 (97.00–116.00)	0.1
Leptin receptor expression (+ cells/mm^2)	1789.47 (668.50–3095.96)	510.76 (206.93–1249.73)	**0.003**
Leptin expression (+ cells/mm^2)	1623.20 (630.34–2627.13)	617.37 (438.00–1049.31)	**0.006**
TGF-β1,2,3 expression (+ cells/mm^2)	953.15 (260.48–1696.27)	1270.70 (579.43–1710.16)	0.37

Data are presented as medians and 25th–75th percentiles. Significance between the 2 studied groups was assessed by using the *Mann-Whitney U test* analysis. The bold represents the significantly differences for P values.

3.3. Leptin parallels Notch-1 expression in turbinate epithelium

To better gain insight into the mechanisms underlying reduced leptin receptor expression, we sought to determinate if the Notch-1 expression parallels with leptin receptor expression. Based on *ex vivo* expression, we observed that leptin receptor might be co-localized with Notch-1 (Fig. 5), and that the expression of both leptin receptor and Notch-1 is decreased in primary turbinate epithelial cells, both *ex vivo* and *in vitro* culture (Figs. 5 and 6 A-B, P = .03 and P = .04, respectively). Leptin receptor is also significantly decreased in total cell extracts from turbinates of allergic patients in comparison to control subjects (P = .001) (Fig. 6 B, P = .001). Furthermore, we performed experiments to assess leptin receptor expression in nasal epithelial RPMI 2650 cells in the presence or absence of the Notch-1 ligand (Jagged-1) alone or in combination with a Notch-1 signaling inhibitor (DAPT). We showed that Jagged-1 significantly increased leptin receptor expression (P = .005) whereas DAPT completely restored it (P = .02) (Fig. 7). Leptin receptor activation using recombinant leptin −1 did not modulate Notch-1 expression (data not shown).

4. Discussion

In chronic nasal disorders, the role of the nasal epithelium in the coordination of inflammatory responses is now well recognized. In this report, using human *in vivo* and *in vitro* experimental approaches, we demonstrate for the first time that the leptin/leptin receptor pathway and Notch-1 expression are both hallmarks of the healthy nasal epithelium of inferior turbinates and that Notch-1 signaling regulates leptin receptor pathway, apparently controlling its expression. Particularly the following new evidence is reported: 1) leptin and leptin receptor expressions are correlated in epithelium from inferior turbinates, are reduced in patients affected by allergic rhinitis and do not co-localize with TGF-β1, 2, 3 expression; 2) *ex vivo*, the expression of leptin receptor seems to co-localize with the Notch-1 expression; 3) primary turbinate epithelial cells extracted from biopsies of allergic patients exhibit lower expression of leptin and Notch-1 receptors; 4) in an *in vitro* model of nasal epithelial cells, leptin receptor expression is induced by the Notch-1 ligand, Jagged-1.

Leptin receptor and its biological ligand leptin are expressed in the nasal mucosal epithelium [6–8,15,18]. This evidence encouraged us to further investigate this pathway. It is well demonstrated that leptin, produced mainly by the adipose tissue, but also by placenta, stomach,

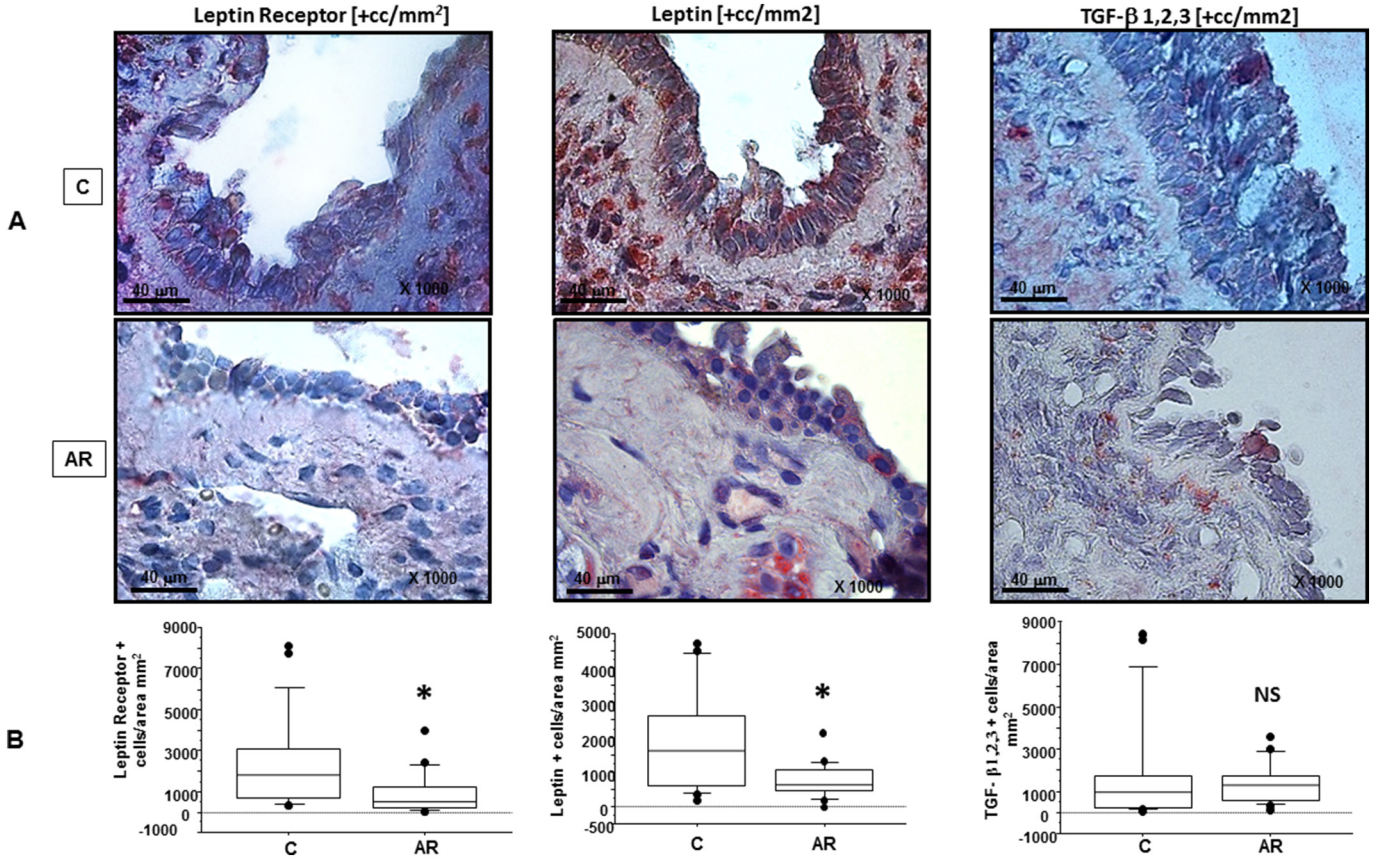

Fig. 1. A. Immunohistochemistry for leptin receptor, leptin and TGF-β1,2,3 in epithelia from inferior turbinate biopsy specimens (red). Healthy subjects (C) and patients with allergic rhinitis (AR) are shown (magnification at X1000, scale bar = 40 µm). B, Leptin receptor and leptin expression are significantly lower* in AR (P = .003 and P = .006 respectively) *versus* the C group whereas TGF-β is not. The results are shown as box-plots with medians (lines inside the boxes), 25th and 75th percentiles (limits of boxes), and the 10th and 90th percentiles (whiskers). NS, not significant. *Mann-Whitney U test* analysis.

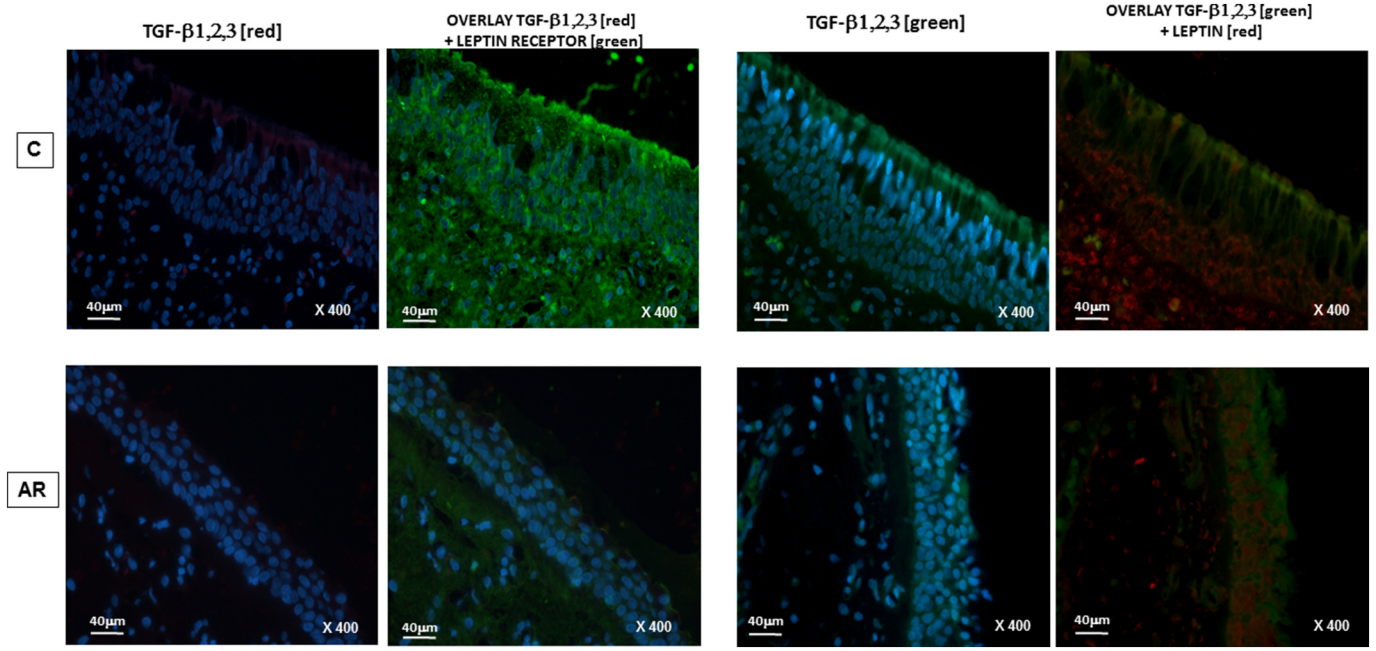

Fig. 2. Immunofluorescence. On epithelium from inferior turbinate biopsy specimens, leptin/leptin receptor and TGF-β1,2,3 expressions are not co-localized. Leptin and its receptor appear more basolateral whereas TGF-β appears more apical (magnification at X400, Scale bar = 40 µm). LEICA DM 4000 microscope.

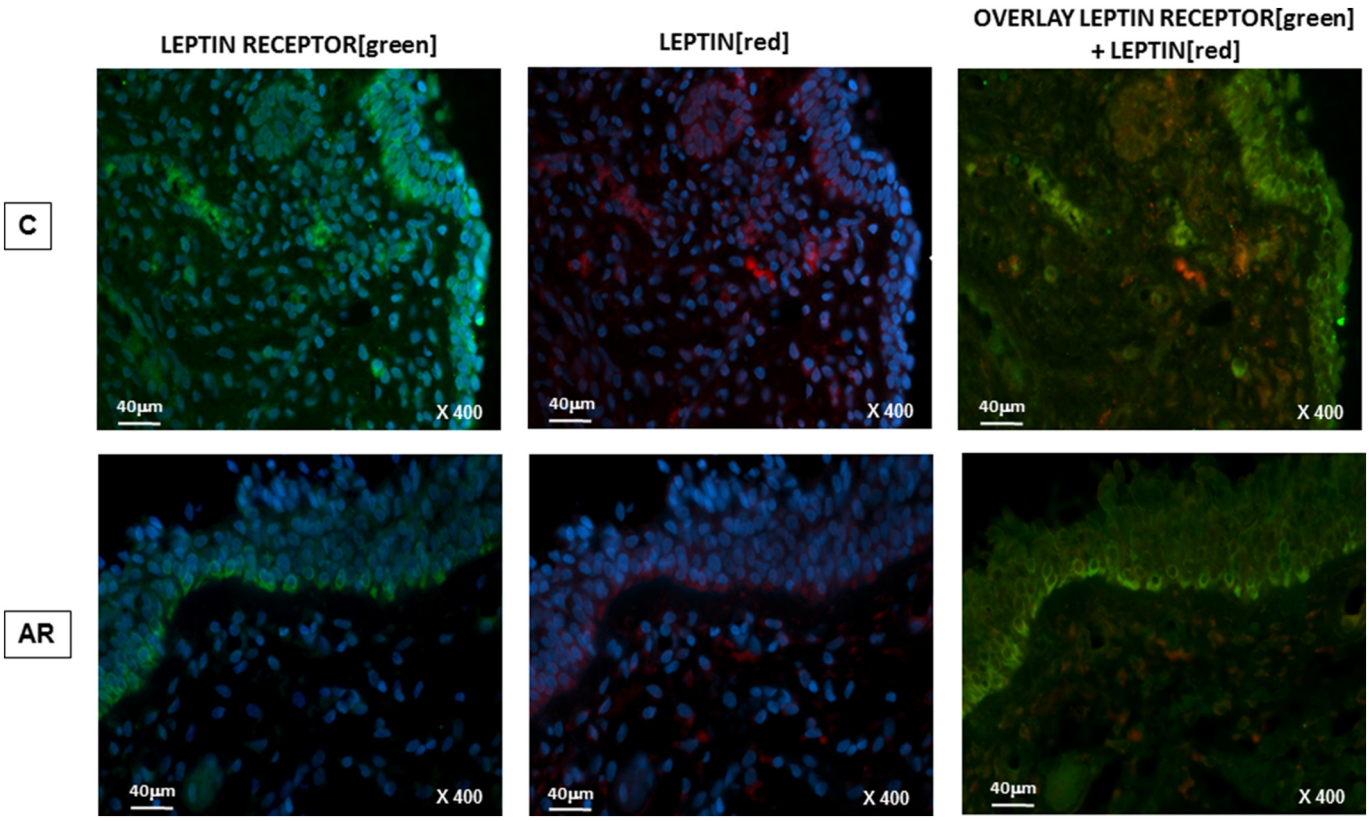

Fig. 3. Immunofluorescence. On epithelium from inferior turbinate biopsy specimens, leptin receptor (green) and leptin (red) are readily detected (magnification at X400, Scale bar = 40 µm) and can be seen to co-localize. Leptin/leptin receptor levels appear diminished in AR as compared to C. LEICA DM 4000 microscope.

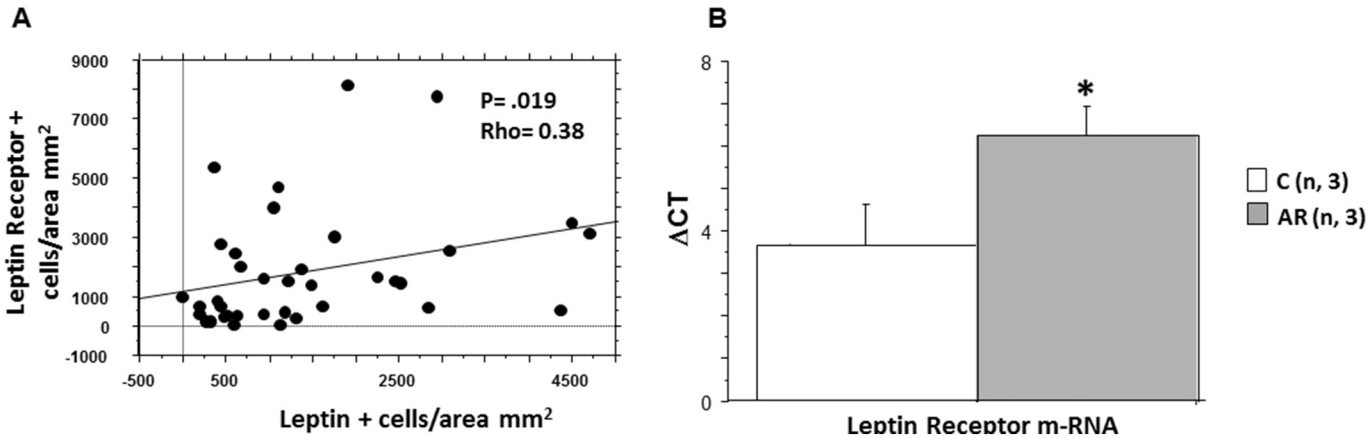

Fig. 4. A. Correlations between expression of leptin receptor and leptin. In epithelia from all inferior turbinates leptin and leptin receptor expression are positively correlated with each other (Rho = 0.38; P = .019). *Spearman's rank correlations*. B. Quantitative real-time PCR of the leptin receptor transcripts is significantly reduced* in AR compared to C (P = .04). *Mann-Whitney U test* analysis. ΔCT is inversely correlated with the gene expression.

fibroblasts, mammary epithelium, and skeletal muscle [19], is involved in the regulation of several systems, from metabolism to immunity, leading to the condition that a genetic leptin deficiency in humans and mice impairs host defenses against respiratory tract infections [20]. Using lung tissues of humans, leptin and its receptor expression has been assessed in bronchial epithelium, in type II pneumocytes and in lipofibroblasts [21–23]. Leptin has a significant role in promoting normal lung maturation: in animals deficient for leptin, daily leptin administration improved respiratory complications, indicating that leptin might act as both a growth factor in the lung and as a neuro-humoral modulator of the central respiratory control mechanisms [22,24]. In the context of inflammation, the pleiotropic hormone leptin is involved in maintaining inflammatory cell survival [25,26]. In cancer, leptin induces cell proliferation and angiogenesis as well as Notch-1 [27,28]. A recent study reports that leptin knockdown could become a new approach for blocking lung cancer progression, which is likely to be mediated, at least partially, by inactivation of the Notch and JAK/STAT3 signaling pathways [29]. In lungs, leptin levels correlated with the severity of lung fibrosis and are increased in patients and mice with acute lung injury by exerting a profibrogenic effect in primary human fibroblasts by augmenting the transcriptional activity of TGF-β1 leading to the suppression of the antifibrotic activity of PPARγ [30,31].

On the other hand, on the side of the epithelium, a functional leptin signaling pathway is present in lung epithelial cells [32] and leptin can be considered a marker for a proliferative effect in epithelial cells, not only in the pathological condition of cancer [18,33,34] but also in

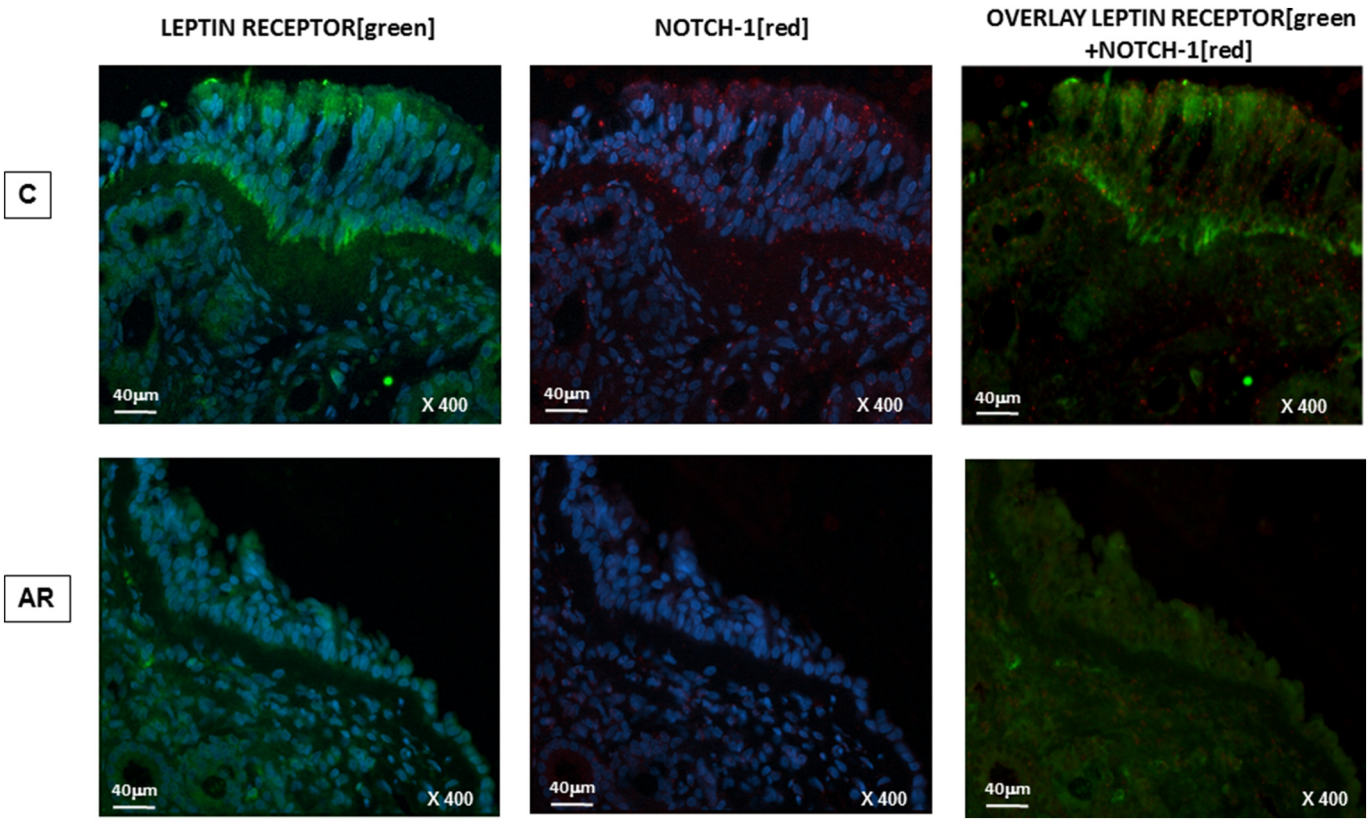

Fig. 5. Immunofluorescence. On epithelium from inferior turbinate biopsy specimens, leptin receptor (green) and Notch-1 (red) are readily detected (magnification at X400, scale bar = 40 μm) and appear to be co-localized. Both markers appear diminished in AR relative to C. LEICA DM 4000 microscope.

patients with asthma and COPD [5,35]. Furthermore, a recent *in vitro* study in human bronchial epithelial cells demonstrated that leptin positively regulates MUC5AC production and secretion induced by IL-13 *via* the JAK2-STAT3 pathway [36]. Thus, leptin seems to have a therapeutic potential for treatment of mucus hypersecretion in chronic inflammatory lung diseases. In addition, it is known that leptin abolishes upper airway obstruction and a recent study demonstrated that intranasal leptin application can bypass leptin resistance, decreased the number of oxygen desaturation events during sleeping, and significantly attenuated sleep-disordered breathing, independently of body weight [37]. Therefore, intranasal administration of leptin may represent a new strategy to treat allergic rhinitis. It should be mentioned, although the role of leptin receptor pathway in lung development, respiratory immune responses and the pathogenesis of inflammatory respiratory diseases is now well established [5,6,21,38], little information has been so far available for the role of this pathway or of Notch-1 signaling in allergic rhinitis.

Regarding leptin and leptin receptor expression, the present study demonstrates that this pathway is reduced in epithelium of inferior turbinates from patients suffering from allergic rhinitis as compared to non-allergic, non-rhinitic subjects. The data herein reported confirmed and extended our previously published results showing with a preliminary *in vivo* approach on a limited number of samples (a total of six), a trend for reduction for leptin and leptin receptor expression in allergic patients in comparison to control subjects [6]. The leptin/leptin receptor pathway plays a homeostatic role in both nasal [6] and bronchial [5] epithelium promoting cell proliferation. The positive regulation of cell proliferation is an event crucially involved in repair processes also in nasal epithelium [39]. Furthermore, corticosteroids contribute to maintaining nasal epithelium homeostasis by up-regulating the leptin/leptin receptor pathway [6]. In contrast, TGF-β1 exerts a direct and an indirect anti-proliferative effect presumably by reducing in human nasal epithelial cells the leptin receptor pathway activity, downregulating both mRNA and protein leptin receptor expression [6]. In addition, in bronchial epithelium of uncontrolled asthmatics, TGF-β1, as well other markers of airway remodeling, inversely correlated with leptin receptor expression [5]. Accordingly, in the present paper, TGF-β1, despite its expression being not significantly reduced in epithelium from inferior turbinates of AR in comparison to C, does not co-localize with leptin and leptin receptor expression.

Based on this evidence, finally we performed further experiments, mainly directed to more deeply investigate Notch-1 signaling in turbinates from AR and C. It is well known that Notch is active in steady state airways, with levels increasing during repair and that it is required for differentiation, but not self-renewal, of airway basal cells [40,41]. Notch signaling links embryonic lung development and asthmatic airway remodeling and, as in different epithelial cytotypes, leptin is also required for the normal maintenance and homeostasis of the intestinal epithelium [42,43]. Notch resides at the cell surface as a single pass transmembrane receptor, transits through the cytoplasm following activation, acts as a transcription factor upon entering the nucleus, interacting with other transcription factors [44]. Jagged-1 Notch ligand, but not Jagged-2 inhibition, induces in the airways of mice a significant decrease in club cells and a corresponding increase in ciliated cells [45]. Regarding Notch-1 expression, the present study demonstrates that it is reduced in primary epithelial cells from inferior turbinates of AR in comparison to C. Furthermore, it appears to co-localize with leptin receptor and Notch-1 signaling activated by the Jagged-1 activating peptide increases levels of leptin receptor itself. This result is in line with previous evidence reported in mouse E0771 breast cancer cells, where DAPT peptide by blocking Notch signaling, abrogated leptin-induced migration, strongly suggesting that leptin-induced proliferation and migration of E0771 cells requires a functional leptin-Notch signaling axis [13].

The findings related to Notch-1 expression are in apparent contradiction with our recently published data demonstrating increased

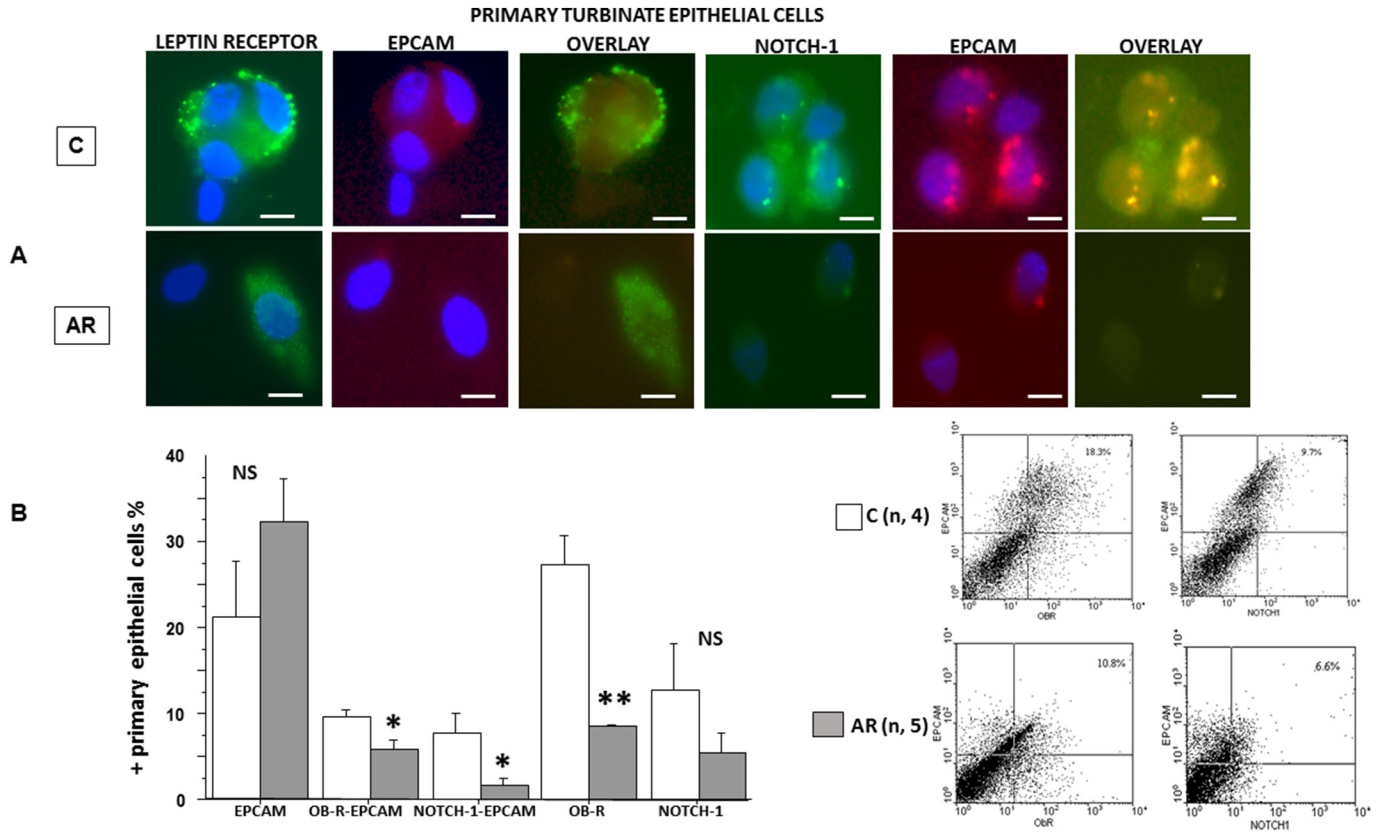

Fig. 6. A. Immunofluorescence for leptin receptor (green) and Notch-1 (red) on primary epithelium obtained from inferior turbinate biopsies from healthy subjects (C, n = 4) and from patients with allergic rhinitis (AR, n = 5) are shown (magnification at X630, Scale bar = 10 μm). Leptin receptor and Notch-1 in EPCAM positive cells appear decreased in AR, but not in C. Axioskop-2-Zeiss microscope. B, Leptin receptor and Notch-1 expression levels in primary turbinate epithelia (EPCAM positive cells) are significantly lower* in AR (P = .03 and P = .04 respectively) than in the C group. Furthermore, the leptin receptor expression in total cell extracts is significantly lower** in AR (P = .001) than in C. NS, not significant. Analysis of variance (*ANOVA*), *Fisher's PLSD* for log data. *Right*, Representative examples of flow cytometric analysis. The numbers indicate the percentage of double positive cells for leptin receptor/EPCAM, and for Notch-1/EPCAM, respectively.

Notch-1 signaling together with increased expression of IL-33 in nasal polyps from allergic subjects [14]. Indeed, nasal polyps are characterized by peculiar structural characteristics and by relevant differentiation abnormalities of the epithelium not present in nasal mucosa from turbinates. So, it is conceivable that the abnormal or delayed reparative response to insult by nasal mucosa owing to down-regulation of homeostatic pathways including the leptin/leptin receptor or Notch-1 signaling, could lead to the activation of other unknown molecular events promoting aberrant and uncontrolled Notch-1 signaling activation promoting differentiation abnormalities (hyperplasia or metaplasia) and to amplify Th2 responses by increasing IL-33 expression. On the other hand, in the inferior turbinate of non-allergic, non-rhinitic subjects, we can recognize a co-localization of Notch-1 together with leptin receptor, that together are down regulated in presence of allergic rhinitis, leading to the concept that both Notch-1 and leptin pathways could represent a hallmark of physiological human nasal epithelial proliferation.

5. Conclusion

The present study provides new evidence for a role for Notch-1 signaling in the regulation of leptin receptor expression in the nasal tract, strongly supporting the concept that Notch-1 signaling and leptin receptor pathway might cooperate to maintain epithelial homeostasis in the upper airways.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dummy.2019.01.002.

Conflict of interest

None

Each of the authors declare that there are no conflicts of interest regarding the publication of this paper.

The manuscript has been read and approved by all authors and all authors agree to the submission of the manuscript to the Journal.

Substantial author contributions

Andreina Bruno: to the conception and design of the study, to the acquisition, the interpretation and the analysis of data and to write the manuscript;

Caterina Di Sano: to the analysis and the interpretation of the data;

Francesco Lorusso: to the conception and design of the study and to the acquisition, the interpretation and the analysis of data;

Paola Dino: to the analysis and the interpretation of the data;

Domenica Russo: to the analysis and the interpretation of the data;

Antonella Ballacchino: to the analysis and the interpretation of the data;

Salvatore Gallina: to the conception and design of the study and to the interpretation of the data;

Domenico Michele Modica: to the interpretation of the data;

Giuseppina Chiappara: to the interpretation of the data;

Hans-Uwe Simon: to the analysis and the interpretation of the data and to revise critically the paper for important intellectual content for final approval of the version to be submitted;

Elisabetta Pace: to the analysis and the interpretation of the data and to revise critically the paper for important intellectual content for

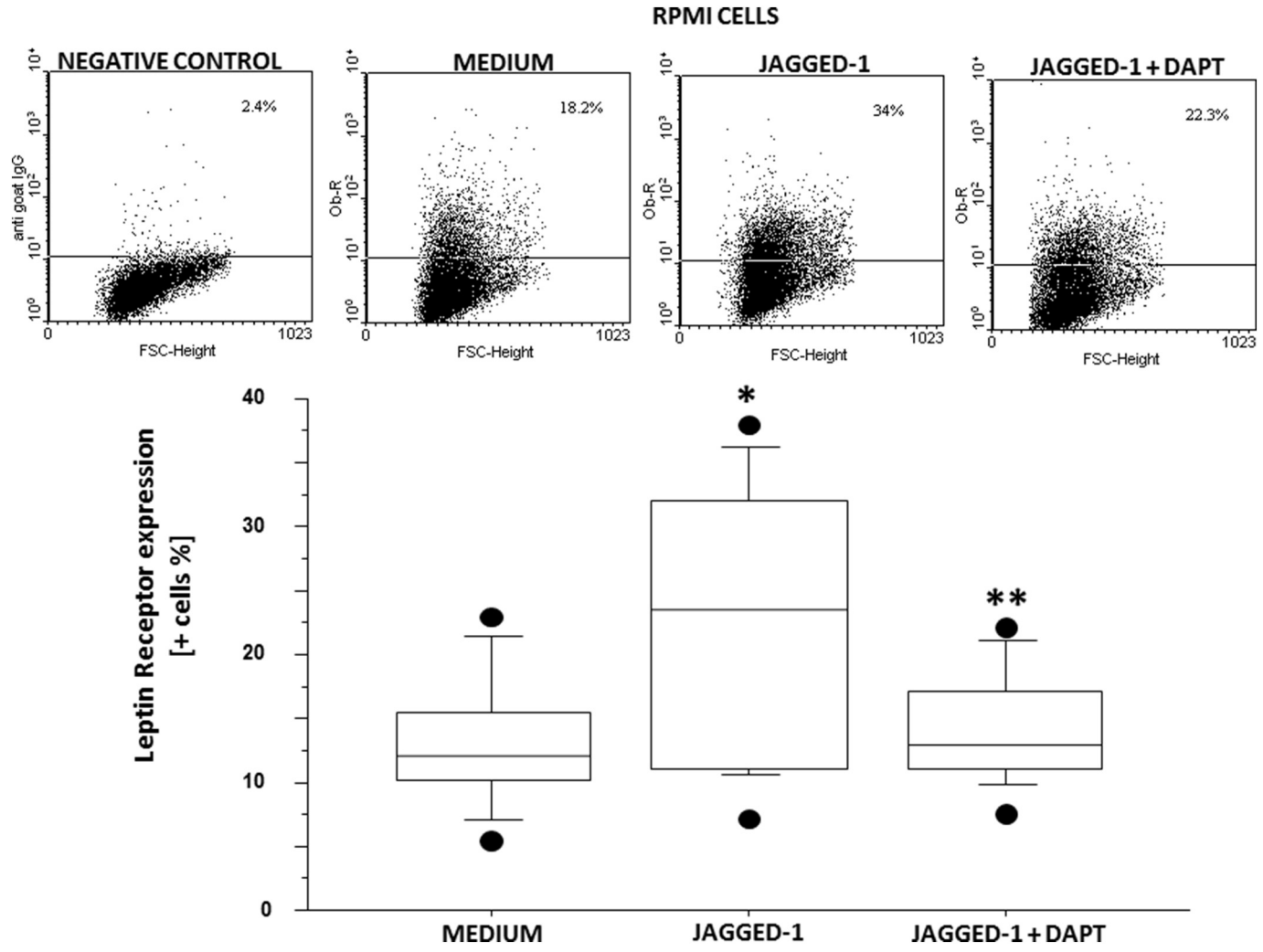

Fig. 7. In the RPMI 2650 cell line, the expression of leptin receptor is significantly increased* (P = .005) by Notch-1 ligand (JAGGED-1, n = 14). Blocking Notch signaling (DAPT, n = 14) restored basal expression of leptin receptor expression and elicited a significantly reduced association ** (P = .02) in comparison to JAGGED-1 alone. The results are shown as box-plots with medians (lines inside the boxes), 25th and 75th percentiles (limits of boxes), and the 10th and 90th percentiles (whiskers). Analysis of variance (*ANOVA*), *Fisher's PLSD* for log data. *Upper*, Representative examples of flow cytometric analysis. The numbers indicate the percentage of leptin receptor positive cells. *FSC-H*, forward light scatter-height.

final approval of the version to be submitted.

Transparency document

The Transparency document associate with this article can be found, in online version.

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