Resistin-Like Molecule α Stimulates Proliferation of Mesenchymal Stem Cells While Maintaining Their Multipotency

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Resistin-like molecule α (RELM α) is highly upregulated in the lungs of mice subjected to hypoxia. It is secreted from pulmonary epithelium and causes potent mitogenic, angiogenic, and vasoconstrictive effects in the lung vasculature. By using bone marrow transplantation in mice, we previously showed that RELM α is able to increase the number of bone marrow-derived cells in lung tissue, especially in the remodeling pulmonary vasculature. The current study investigated the effect of RELM α on progenitor stem cell content in mouse lung. Hypoxia, while stimulating RELM α expression, caused an increase in the number of Sca1⁺/CD45⁻ progenitor cells in lungs of wild-type mice, but not in lungs of RELM α knockout mice. An *in vitro* study with cultured mesenchymal stem cells (MSCs) showed that RELM α induced a robust proliferative response that was dependent on Phosphatidylinositol 3-kinase/Akt and Erk activation. RELM α treatment of MSCs caused upregulation of a large number of genes involved in cell cycle, mitosis, organelle, and cytoskeleton biogenesis, and DNA metabolism. MSCs cultured in RELM α -supplemented media were able to maintain their differentiation potential into adipogenic, osteogenic, or mesenchymal phenotypes, although adipogenic differentiation was partially inhibited. These results demonstrate that RELM α may be involved in stem cell proliferation in the lung, without affecting differentiation potential.

Introduction

COMPELLING EVIDENCE SUGGESTS that bone marrow-derived stem cells are recruited to the lungs in a variety of respiratory diseases. However, the factors and molecular mechanisms that regulate the biology of these stem cells during specific respiratory diseases have only begun to be explored. Further, the contribution of bone marrow-derived cells to a specific disease progression is not completely clear. We hypothesized that Resistin-like molecule α (RELM α), a protein expressed in the lung during a variety of disease states, may be involved in stem cell proliferation.

RELM α is a member of the resistin family of proteins. In normal mouse lung, RELM α expression is low, but it is greatly increased in hypoxia-induced pulmonary hypertension [1], allergic airway inflammation [2], bleomycin-induced lung fibrosis [3], and asthma [4]. RELM α is expressed by macrophages and pulmonary epithelial cells [2] and also by pulmonary vascular cells during hypoxia [1]. The Th-2 cytokines IL-4 and IL-13 induce RELM α expression via STAT6

and JAK-1 pathways [3,5]. RELM α has the capacity to promote lung cell proliferation, angiogenesis, and inflammation [1,6]. Its expression is an indicator of macrophage activation [7,8]. RELM α has antiapoptotic effects on embryonic lung explants [9] and lung fibroblasts [10]. It also has chemokine actions and causes the upregulation of VEGF, VEGFR2, SDF-1, and MCP-1 in the remodeling hypoxic lung model and *in vitro* [6]. Finally, RELM α can induce myofibroblast differentiation in lung fibroblast culture [3].

Although the pivotal role of the resistin family of cytokines has been established in many pathophysiological processes, almost nothing is known about their role in stem cell physiology. However, being a lung-specific protein, RELM α may affect stem cell fate in the lung during hypoxia or other pathological conditions. Recent studies have suggested that bone marrow-derived cells have the capacity to produce nonhematopoietic derivatives that participate in the regeneration and repair of diseased adult organs, including lung [11]. Chronic hypoxia is a common cause of pulmonary hypertension and pulmonary vascular remodeling. Using two

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neonatal animal models (rat and calf) of chronic hypoxic pulmonary hypertension, Frid et al. [12] demonstrated that hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal precursors of a monocyte/macrophage lineage. In a study of hypoxia-induced pulmonary hypertension in mice, bone marrow-derived cells were mobilized to the hypertensive pulmonary arteries where they acquired smooth muscle phenotype and contributed to the pulmonary vascular remodeling [13].

Data from our laboratory show that RELM α increases the number of bone marrow-derived cells in the vasculature of mouse lung [14]. These cells are positive for the stem cell markers sca-1 and c-kit and the smooth muscle marker α -smooth muscle actin (α -SMA) and are negative for the endothelial cell markers CD34 and CD31. Further, we demonstrated that RELM α induces migration of primary cultured murine bone marrow cells [15] and human mesenchymal stem cells (MSCs) [14]. Thus, as an activator of bone marrow cell migration, RELM α may be critical to pulmonary vascular remodeling. In the current study, we investigated the role of RELM α in adult stem cell fate.

Materials and Methods

Reagents and antibodies

Recombinant mouse RELM α was purified from stably transfected HEK-293 cells as described previously [1]. Phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 and MEK inhibitor U0126 were purchased from EMD Biosciences. SuperFasLigand[™] (FasL) was purchased from Enzo Life Sciences. The following antibodies were used: phospho-p38 MAPK (Thr180/Tyr182) mouse mAb, phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) rabbit mAb, Egr-1 rabbit mAb, phospho-Akt, phospho-p65 (Ser536) rabbit mAb, adiponectin rabbit mAb, and β -Tubulin rabbit mAb (Cell Signaling Technology); β -actin mouse mAb clone AC-15 and GAPDH mouse mAb (Sigma-Aldrich); RELM α goat antibody (R & D Systems); osteopontin mouse mAb (Abcam), fibronectin mouse mAb, fluorescein isothiocyanate (FITC)-labeled rat anti-mouse Ly-6A/E (Sca-1) Ab, and FITC-labeled rat anti-mouse CD45 Ab (BD Biosciences).

Experimental animals and hypoxic treatment

Twelve-week-old female *Retnla*^{-/-} mice (backcrossed to BALB/c background) generated by Regeneron Pharmaceuticals were a gift from Marc Rothenberg with permission from Regeneron Pharmaceuticals. Control female BALB/c mice were purchased from Charles River Laboratories. All mice were housed under specific pathogen-free conditions and all uses were approved by the Animal Care and Use Committee of the Johns Hopkins University. The mice were exposed to either normal room air (20.8% O₂) or hypoxia (10% O₂) for 28 days. Then mice were sacrificed and the lung tissue processed as stated below.

Pulmonary cell isolation and flow cytometry analysis

Mice were euthanized by isoflurane overdose, and the lungs were perfused with phosphate buffered saline (PBS) via the right heart. Lungs were dissected and minced into 1–2-mm pieces in a digestive solution containing 250 U/mL

collagenase I (Worthington, CLSS-1) and 50 U/mL DNase (Sigma, D4263) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. The suspension was incubated on a shaker for 30 min at 37°C and then strained through a 40- μ m cell strainer and centrifuged for 10 min at 700 rpm. The pellets were resuspended with 1 mL of DMEM, passed through a 20- μ m cell strainer, and then divided into aliquots, which were centrifuged again in 5-mL polystyrene tubes for 5 min at 700 rpm. The supernatant was removed, and the samples were blocked with FC Block (BD Bioscience, 553140) at a 1:10 dilution [in 5% bovine serum albumin (BSA)] for 30 min at 4°C. The samples were stained with FITC CD45 (BD Biosciences, 553080) and PE Ly-6A/E (Sca-1; BD Biosciences, 553108) at a 1:40 dilution (in 5% BSA) for 30 min at 4°C. Samples were washed thrice with cold PBS. After the pellets were resuspended in 400 μ L of PBS, 5 μ L of 7-AAD was added to each tube. Samples were analyzed on a BD FacScan flow cytometer for 7-AAD^{neg}, CD45^{neg}, and ScaI^{pos}. Data were processed with CellQuest software (BD Biosciences).

Cell culture and treatment

Human bone marrow MSCs from Lonza were cultured to passage 4 or earlier in MSC growth medium (Lonza). For the proliferation assay cells were stimulated with vehicle or 100 nM RELM α in serum-free medium for 24 h under normal or hypoxic condition (2% O₂). Bromodeoxyuridine (BrdU) was simultaneously added with the stimuli. Proliferation was analyzed by BrdU assay (EMD Biosciences) according to the manufacturer's protocol.

Adipogenic or osteogenic differentiation was induced by culturing the cells in adipogenic medium (Lonza) for 3 weeks or in osteogenic medium (Lonza) for 2 weeks, in the absence or presence of 100 nM RELM α . Calcium phosphate deposition was stained with Alizarin Red. Cells were rinsed with PBS, fixed in ethanol for 30 min, stained in 2% aqueous Alizarin Red solution for 5 min at room temperature, washed with water, and air dried. Lipid deposition was assessed using Oil Red O staining. The cells were fixed with 10% formalin for 30 min, rinsed with PBS, incubated with 60% isopropanol for 5 min, then stained with Oil Red O solution in 60% isopropanol for 5 min. Cells were rinsed with water and analyzed under the microscope. Lipid deposition and calcium deposition were quantified using ImageJ software by calculating cell surface area occupied by the staining. The data are presented as pixels per microscopic field $\pm$ SE ($n = 5-6$).

For western blot analysis, cells grown in 6-well dishes were stimulated with either vehicle or 100 nM RELM α for different time periods. Then the medium was removed and cells were lysed with 300 μ L of Laemmli sample buffer and analyzed by western blot. Apoptosis was assayed by flow cytometry with the PE Annexin V Apoptosis Detection Kit (BD Pharmingen).

Microarray analysis

For the gene array study, subconfluent cells were treated with either vehicle or 100 nM RELM α for 48 h. Each group (vehicle or RELM α treatment) consisted of six 60-mm dishes. Total RNA was extracted using the TRIzol Reagent method. Additional purification was performed on RNeasy

columns (Qiagen). The quality of total RNA samples was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies).

RNA samples were labeled according to the chip manufacturer's recommended protocols. In brief, for Illumina, 0.5 µg of total RNA from each sample was labeled by using the Illumina TotalPrep RNA Amplification Kit (Ambion) in a two-step process of cDNA synthesis followed by in vitro RNA transcription. Single-stranded RNA (cRNA) is generated and labeled by incorporating biotin-16-UTP. 0.75 µg of biotin-labeled cRNA is hybridized (16 h) to Illumina Sentrix HumanRef8 V3 BeadChips (Illumina). The hybridized biotinylated cRNA was detected with streptavidin-Cy3 and quantified using Illumina's BeadStation 500GX Genetic Analysis Systems scanner. Preliminary data analysis of the scanned data was performed using Illumina BeadStudio software, which returns single intensity data values/gene following the computation of a trimmed mean average for each probe type represented by a variable number of bead probes/gene on the array. Significant gene lists were calculated by selecting genes that satisfy a significance threshold criteria of P -values ≤ 0.001 , a false discovery rate ≤ 0.1 , and a twofold change or greater. Upregulated genes were submitted to the DAVID database (<http://david.abcc.ncifcrf.gov>), which clusters genes by function according to a series of common keywords [16]. The proportion of each keyword in the list is compared with that in the whole genome, making it possible to compute P -values and enrichment scores (geometric mean of the inverse log of each P -value).

FIG. 1. Resistin-like molecule α (RELMA) expression correlates with increased content of nonhematopoietic progenitor cells in the lung. **(A)** Genomic polymerase chain reaction shows that RELMA DNA is expressed in wild-type (WT), but not in RELMA knockout (KO) mice. **(B)** Western blot of RELMA expression in WT and KO mice after 4 days of hypoxia challenge. **(C)** WT and RELMA KO mice were subjected to either normal or hypoxic conditions for 4 weeks; then lung cells were analyzed by flow cytometry for the presence of Sca1⁺/CD45⁻ nonhematopoietic progenitor cells. Values are shown as mean $\pm$ SEM ($n = 4-8$) relative to the total number of cells (defined as 100%); * $P = 0.004$ and ** $P = 0.03$.

TABLE 1. SELECTED CELL CYCLE-RELATED GENES UPREGULATED MORE THAN 3 TIMES ($P < 0.001$)

Gene symbol	Definition	Fold change
UBE2C	Ubiquitin-conjugating enzyme E2C (UBE2C), transcript variant 3	4.79
CDC20	CDC20 cell division cycle 20 homolog (S. cerevisiae) (CDC20)	4.73
PBK	PDZ-binding kinase (PBK)	4.44
HMMR	Hyaluronan-mediated motility receptor (RHAMM) (HMMR), transcript variant 1	4.34
BIRC5	Baculoviral IAP repeat-containing 5 (survivin) (BIRC5), transcript variant 1	4.27
CDCA5	Cell division cycle associated 5 (CDCA5)	4.03
CDCA3	Cell division cycle associated 3 (CDCA3)	3.81
TYMS	Thymidylate synthetase (TYMS)	3.81
TOP2A	Topoisomerase (DNA) II alpha 170 kDa (TOP2A)	3.78
AURKA	Aurora kinase A (AURKA), transcript variant 3	3.74
CEP55	Centrosomal protein 55 kDa (CEP55)	3.73
NUSAP1	Nucleolar and spindle associated protein 1 (NUSAP1), transcript variant 1	3.73
AURKB	Aurora kinase B (AURKB)	3.69
KIF20A	Kinesin family member 20A (KIF20A)	3.66
PRC1	Protein regulator of cytokinesis 1 (PRC1), transcript variant 1	3.65
CDKN3	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase) (CDKN3)	3.6
CDC2	Cell division cycle 2, G1 to S and G2 to M (CDC2), transcript variant 2	3.5
CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae) (CDC45L)	3.48
CCNB2	Cyclin B2 (CCNB2)	3.43
TK1	Thymidine kinase 1, soluble (TK1)	3.43
CENPF	Centromere protein F, 350/400ka (mitosin) (CENPF)	3.37
CCNB1	Cyclin B1 (CCNB1)	3.3
CENPM	Centromere protein M (CENPM), transcript variant 2	3.21
CENPA	Centromere protein A, 17 kDa (CENPA)	3.19
TTK	TTK protein kinase (TTK)	3.17
POLQ	Polymerase (DNA directed), theta (POLQ)	3.1
KIF11	Kinesin family member 11 (KIF11)	3.05

TABLE 2. FUNCTIONAL ANNOTATION CHART OF RESISTIN-LIKE MOLECULE α -UPREGULATED GENES IN HUMAN MESENCHYMAL STEM CELL

Cellular function	Number of genes	P value ^a
Cell cycle	69	8.0E-55
Cell cycle process	56	2.8E-45
M phase	50	4.6E-49
Cell division	44	6.3E-48
Mitosis	41	3.6E-43
Nuclear division	41	3.6E-43
Organelle fission	41	2.0E-42

^aThe enrichment P-value is calculated based on EASE Score, a modified Fisher Exact Test (see [ref. 38]).

Results

RELM α expression correlates with increased content of nonhematopoietic progenitor cells in the lung

We have previously shown that both chronic hypoxia and RELM α gene transfer in mouse lungs increases the number of bone marrow-derived stem cells in and around the pulmonary vasculature [14]. Here we used RELM α knockout mice to investigate whether expression of RELM α affects progenitor cell content in the lungs. The knockout mice lack RELM α gene (Fig. 1A) and protein expression (Fig. 1B), while wild-type mice have strong expression of RELM α in the lungs after hypoxic exposure (Fig. 1B). The knockout mice had less Sca1⁺/CD45⁺ progenitor cells in the lungs than wild-type mice in both control- and hypoxic conditions (Fig. 1C). Hypoxia, however, while produced an increase in the number of Sca1⁺/CD45⁺ cells in the lungs of both groups of mice, had stronger effect in wild-type animals (Fig. 1C). The difference in progenitor cell content in the lungs of wild-type versus knockout mice under normoxic conditions can be explained by the fact that while undetectable by western blot, a small amount of RELM α is still expressed under normoxia in the lungs of wild-type mice, as evidenced by immunohistochemistry [14].

RELM α induces genes related to cell division cycle in MSCs

Since RELM α was shown to cause proliferation of several cell types [1,6], it may be responsible for the increased proliferation of stem cell progenitors in the lung during hypoxia. We next examined whether RELM α induces a mitogenic/proliferative response in multipotent MSCs. A gene array study showed that multiple genes associated with cell division cycle were upregulated in MSCs after RELM α treatment (Table 1). Functional analysis of RELM α -affected genes in MSCs by the DAVID Functional Annotation Tool [16] showed that genes upregulated by RELM α are involved in cell cycle, mitosis, and organelle biogenesis (Table 2).

RELM α activates several pro-growth signaling pathways

RELM α activated signaling pathways associated with mitogenic response, such as Erk MAPK, and Akt, but not p38 MAPK or NF κ B, which are usually associated with differentiation and apoptosis (Fig. 2A). Additionally, RELM α stimulation caused nuclear expression of Egr-1 (early growth response protein; Fig. 2B, C), a zinc finger transcription factor that has a key role in mediating proliferative responses [17].

In accordance with gene expression data, direct quantification with the BrdU incorporation assay showed that RELM α significantly stimulated cell proliferation. This proliferation was inhibited by LY294002 (PI3K inhibitor) and by U0126 (MEK1/2 inhibitor; Fig. 3A). Figure 3B shows that LY294002 and U0126 successfully blocked the corresponding signaling pathway. Since the expression of RELM α is upregulated by hypoxia, and the number of progenitor cells is increased in mouse lung after hypoxia exposure, we have tested whether RELM α is able to induce cell proliferation in hypoxic environment in vitro. We found that hypoxia alone promotes MSC proliferation, which has been reported previously [18,19]. However, RELM α is still able to induce MSC proliferation even further (Fig. 3C).

RELM α does not prevent MSC apoptosis

Despite causing a strong proliferative response, RELM α was not effective in preventing MSC apoptosis induced by serum starvation or by FasL (Fig. 4).

FIG. 2. RELM α -induced activation of intracellular signaling in mesenchymal stem cells (MSCs). Cells were treated with 100 nM RELM α for the times indicated. Tumor necrosis factor α (10 ng/mL) was used as a control. Phosphorylation of Akt, Erk, p38, and NF κ B (p65 RelA) (A) and expression of Egr-1 (B) were analyzed by western blot. RELM α induced Akt and Erk1/2 phosphorylation and Egr-1 expression in MSCs. (C) Immunofluorescent staining of Egr-1 shows increased expression in the nuclei 1 h after stimulation with RELM α . Color images available online at www.liebertpub.com/scd

FIG. 3. RELM α stimulates proliferation of MSCs in both normoxic and hypoxic conditions. **(A)** Cells were pretreated with PI3K inhibitor LY 294002 (10 μ M) or MEK inhibitor U0126 (5 μ M) for 30 min before RELM α stimulation. BrdU incorporation assay shows significant stimulation of cell proliferation by RELM α (100 nM) that was inhibited by LY294002 and by U0126, * P < 0.05 compared with vehicle. **(B)** Western blot analysis shows that LY294002 abolishes RELM α -induced Akt phosphorylation and that U0126 abolishes RELM α -induced Erk1/2 phosphorylation. **(C)** BrdU incorporation assay at hypoxic conditions (1% O₂) shows that RELM α (100 nM) stimulates cell proliferation (** P < 0.05 RELM α vs. Control; *** P < 0.05 Hypoxia vs. Normoxia). BrdU, bromodeoxyuridine.

FIG. 4. RELM α does not inhibit apoptosis in MSCs. Cells were grown in complete growth medium. Apoptosis was induced by Super-FasLigand (FasL; 10 ng/mL) or by serum starvation for 24 h in the absence or presence of RELM α (100 nM). Apoptotic cells were identified by flow cytometry as Annexin V^{pos}7-AAD^{neg} (lower enclosed area).

RELM α -treated MSCs maintain their differentiating potential

Generally, proliferation and differentiation are inversely correlated processes during tissue development and homeostasis. Because RELM α is such a potent mitogenic factor for MSCs, we investigated whether it inhibits their differentiation. MSCs were cultured in adipogenic or osteogenic medium, or in the presence of transforming growth factor β 1, which induces myofibroblast-like differentiation and stimulates expression of α -actin in MSCs [20]. Such myogenic differentiation may be relevant in the lung environment, since we and others showed coexpression of stem cell markers with SMA in stem cells in the lung [14,21]. RELM α partially inhibited adipogenic differentiation, as evidenced by reduced lipid accumulation (Fig. 5A) and adipocyte marker expression

FIG. 5. Effect of RELM α on adipogenic differentiation of MSCs. MSCs were stimulated to induce differentiation in the absence or presence of 100 nM RELM α . **(A)** Oil Red O staining of lipids in MSCs stimulated for 4 weeks with either adipogenic or growth medium. Oil Red O staining was quantified using ImageJ software (* $P < 0.05$). **(C)** Western blot shows the expression of adipogenic markers PPAR γ and adiponectin in MSCs stimulated for 4 weeks with either adipogenic or growth medium.

(Fig. 5B). RELM α did not affect differentiation into osteogenic (Fig. 6A, B) or myofibroblast phenotypes (Fig. 6C).

Discussion

We have previously shown that RELM α contributes to vascular remodeling during hypoxia-induced pulmonary

FIG. 6. Effect of RELM α on osteogenic and myogenic differentiation of MSCs. **(A)** Osteogenic differentiation was stimulated for 3 weeks with osteogenic medium, cells were stained with Alizarin Red for calcium phosphate deposits. The staining was quantified using ImageJ software (* $P = 0.02$). **(B)** Western blot shows the expression of osteopontin. **(C)** Myogenic differentiation was induced with 10 ng/mL transforming growth factor β 1 in growth medium for 5 days. Western blot shows the expression of smooth muscle actin and fibronectin as markers of myofibroblasts.

hypertension [22] and acts as a chemotactic molecule to stimulate the migration of myeloid cells through activation of the Bruton's tyrosine kinase pathway [15]. Further, using a bone marrow transplantation model, we have shown that pulmonary-specific overexpression of RELM α increases the number of bone marrow-derived cells directly engrafted into the vascular wall [14]. Here, we used RELM α knockout mice to quantitatively show that progenitor cell content in the lungs positively correlates with RELM α expression. Further, we found that MSCs cultured in vitro exhibited a robust proliferative response to RELM α that was dependent on PI3K/Akt and Erk activation. Although adipogenic differentiation was partially inhibited, osteogenic and myogenic differentiation was unaffected.

The importance of stem cells for basic lung function and for the treatment of respiratory diseases has now been recognized. Bone marrow contains classical hematopoietic and other types of stem cells, including mesenchymal and endothelial progenitor cells. Pulmonary remodeling may involve proliferation of resident pulmonary cells or recruitment of stem cells to the lung. Stem cells from circulating blood contribute to lung repair in several models of injury, including models produced by lipopolysaccharide [23,24], elastase [25,26], irradiation [27,28], naphthalene [29], and bleomycin [30]. Importantly, bone marrow-derived cells mobilize to pulmonary arteries and contribute to the vascular remodeling in hypoxia-induced pulmonary hypertension [13,14].

RELM α has been shown to induce proliferation of vascular smooth muscle cells and endothelial cells [1,31]. Our previous work suggested that RELM α also exerts a chemokine effect on bone marrow progenitor cells. RELM α stimulated migration of bone marrow cells in vitro [14,15] and pulmonary RELM α gene transfer via adeno-associated viral vector increased the number of bone marrow-derived cells in the lungs [14]. Our present data reveal that RELM α exerts a strong mitogenic effect on stem cells in vitro. We studied RELM α -induced proliferation 24 h after the stimulation, and gene array showed mitogenic response 48 h after the stimulation. It is possible, therefore that some indirect effects of RELM α , such as autocrine stimulation via growth factor release, may be responsible for the stimulation. Our gene array, however, did not reveal any RELM α -dependent upregulation of cytokine/growth factor genes.

It has been a longstanding discussion whether pulmonary stem progenitor cells are recruited from the bone marrow during lung vascular remodeling or whether they proliferate directly in the lung. Although our current work cannot answer this question, it clearly shows that in addition to its chemokine action, RELM α is a potent mitogenic stimulus for bone marrow MSCs. Whether RELM α plays a role in phenotypic change of these cells in the lung remains unknown.

Human bone marrow expresses high levels of resistin, another member of the RELM family [32]. It is possible to speculate that resistin plays a significant role in bone marrow proliferation and homeostasis. Interestingly, Bentley et al. [33] showed that the number of multipotent mesenchymal stromal cells increased in the lungs of mice subjected to an ovalbumin-induced asthma model. It is also known that RELM α expression is highly upregulated in mouse lungs

when subjected to that asthma model [2]. Therefore, the proliferation of multipotent mesenchymal stromal cells may be explained by RELM α -dependent chemotaxis and recruitment of circulating stem cells into the lung, as we previously reported [14] and by increased proliferation of stem cell progenitors within the lung.

RELM α -induced proliferation of MSCs is Erk- and PI3K-dependent. Activation of PI3K/Akt pathways has been implicated in inhibition of apoptosis [34]. We, however, failed to detect any antiapoptotic effect of RELM α on MSCs, although such an effect has been previously shown on fibroblasts [10] and in the lung during development [9].

The role of resistin in adipogenesis has been extensively studied. However, the results are controversial, as both inhibitory [35,36] and stimulatory effects [37] have been reported. Here, we found that RELM α partially inhibited adipogenic differentiation but did not affect osteogenic or mesenchymal differentiation.

In conclusion, this study demonstrates that RELM α expression correlates with the number of nonhematopoietic progenitor cells in the mouse lung in vivo and that RELM α stimulation causes a potent mitogenic response in MSCs in vitro.

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Author Disclosure Statement

There is no conflict of interest.

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