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Comparative studies of resistin expression and phylogenomics in human and mouse

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Abstract

Resistin is a newly identified adipocytokine that has been proposed to be a link between obesity and type 2 diabetes based on animal studies. However, the role of resistin in the pathogenesis of insulin resistance associated with obesity in humans remains unclear. We comparatively and quantitatively studied the tissue distributions of resistin mRNA between human and mouse. The expression level of resistin mRNA in human adipose tissue is extremely low but detectable by real-time PCR and is about 1/250 of that in the mouse. Remarkably, resistin mRNA is abundant in human primary acute leukemia cells and myeloid cell lines U937 and HL60, but not in the Raw264 mouse myeloid cell line. Resistin expression in U937 cells was not affected by lipopolysaccharide (LPS) or by ciglitazone, a PPARγ ligand. Phylogenomics revealed that the human resistin gene is the ortholog of its murine counterpart and is located in a region of chromosome 19p13.3, which is syntenic to mouse chromosome 8A1. In addition to the resistin-like molecule (RELM) sequences already reported, bioinformatics analysis disclosed another RELM sequence in the vicinity of RELMβ on human chromosome 3q13.1, but this sequence is unlikely to encode an expressed gene. Therefore, only two RELMs, resistin and RELMβ, exist in humans, instead of the three RELMs, resistin, RELMα, and RELMβ, that exist in mice. This finding provides a possible answer to the question of why only two RELMs have been cloned in humans and suggests that the RELM family is not well conserved in evolution and may function differently between species. Therefore, caution should be exercised in interpreting resistin as a link between obesity and insulin resistance in humans. The high expression of resistin in human leukemia cells suggests a hitherto unidentified biological function of resistin in leukocytes.

Obesity is closely associated with insulin resistance and type 2 diabetes, both in rodents and humans; but the underlying mechanism is poorly understood. Adipose tissue has been a focus of research for the mechanism because it secretes many bioactive substances that regulate insulin sensitivity and energy metabolism and also is the principal target tissue of the antidiabetic thiazolidinediones (TZDs), which are ligands for the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ).

Resistin is a new adipocytokine discovered from the systematic screening of adipose genes that were down-regulated by TZDs [1]. The initial study indicated that resistin expression was increased in obesity and suppressed by TZDs and that recombinant resistin inhibited insulin-mediated glucose uptake by 3T3-L1 adipocytes and impaired glucose tolerance in mice. Hence, this molecule has been proposed as a link between obesity and insulin resistance.

However, subsequently, conflicting experimental results regarding the role of resistin in obesity-induced insulin resistance have been reported. In favor of the proposed role, the infusion of recombinant resistin and its homolog RELMβ rapidly induced hepatic insulin resistance in mice [2], and overexpression of mouse resistin inhibited glucose uptake by mouse L6 skeletal muscle cells [3], thus demonstrating that resistin does promote insulin resistance. Conversely, other studies have suggested that resistin may not be involved in

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obesity-associated insulin resistance. These studies found that resistin mRNA expression is decreased in several obese animal models, and is induced, rather than inhibited, by TZD treatment [3,4]. In addition, several known insulin resistance-inducing factors, such as free fatty acids (FFA) [5], high-fat diet [6], and TNFα [7], reduce resistin expression. Significantly, the expression of human resistin is very low in fat tissue and does not correlate with obesity or diabetes [8]. These observations have raised the question of whether resistin is an important link between obesity and insulin resistance [9] and whether human resistin is the evolutionary ortholog of mouse resistin.

Resistin is a 12.5-kDa peptide belonging to the family of RELMs (resistin-like molecules) [10] or FIZZ (found in inflammatory zone) proteins [11], characterized by a cysteine-rich motif at the carboxyl terminus. In mice, this family consists of three members: resistin (FIZZ3), RELMα (FIZZ1), and RELMβ (FIZZ2), each with a distinct tissue distribution. Resistin is highly expressed in adipocytes, RELMα is expressed in the adipose stromal fraction and lung, and RELMβ is expressed in colon and intestine. A significant feature of this protein family is the relatively low conservation at the protein level among the family members both inter-species (33–59% identity) and intra-species (27–51% identity) [11]. So far, only two RELMs, resistin and RELMβ, have been reported in humans. This somewhat complicates the elucidation of the role of resistin in human pathophysiology [12,13] since there is always the possibility of the existence of a third, yet-to-be cloned RELM in humans that may behave more like murine resistin in expression and biology.

Comparative biology is a powerful approach to understand the conservation of biological function among species. Thus, we comparatively studied resistin expression and conducted phylogenomics studies of the RELM family. We report here that human resistin is the ortholog of its murine counterpart but with a dramatic difference in gene expression, particularly in adipose tissue. We also provide evidence that, unlike in mice, only two RELMs are expressed in humans. These results suggest that significant divergence occurred during evolution of the RELM family and that caution should be exercised when extrapolating the insights from the mouse resistin studies into humans.

Research design and methods

Oligonucleotide and cDNA sequences. All oligonucleotides were synthesized by the Biopolymer Laboratory of the University of Maryland and are listed in Table 1. The PCR primer pairs were designed across exon(s) to avoid false positive signals from potentially contaminating genomic DNA. The gene-specific primers used for real-time PCR were designed such that they are comparable with regard to the sequence position and melting temperatures between the species, and were tested to have yielded single expected amplicons by conventional PCR. PCR amplicons were verified by sequencing with BigDye (Applied Biosystems, Foster City, CA).

Sample collection and Northern blot analysis. Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) at the age of 7–9 weeks were euthanized by CO_2 and the tissues of interest were excised and snap-frozen in liquid nitrogen. Human omental and subcutaneous fat tissues were obtained from biopsy or autopsy samples from the National Disease Research Interchange (NDRI, Philadelphia, PA) through an IRB-approved protocol. All other human tissue RNAs were purchased from BD Bioscience (Palo Alto, CA). Human U937 and HL60 and mouse Raw264 cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA) and cultured according to the manufacturer's instructions. For differentiation into monocytes/macrophages, U937 cells were treated with 1 μM phorbol myristate acetate (PMA) (Sigma, St. Louis, MO) and allowed to adhere for 48 h, after which they were fed with PMA-free medium and cultured for 24 h

Table 1
Oligonucleotide sequence, location, and application

Gene	Accession No. (location)	Name	Sequence	Application
mResistin	AF323080 (nt 46–455)	G1150 G1151	5′-GTTGTGTCCTGCTAAGTCCT-3′ 5′-CTGGAAACCACGCTCACTTC-3′	Real-time PCR Northern*
hResistin	AF205952 (nt 1–410)	G1152 G1153	5′-AGCCCACCGAGAGGCGCCTG-3′ 5′-GCAACCCCTCCGGACCTGGA-3′	Real-time PCR Northern
mCyclophilin	BC013713 (nt 144–663)	G1373 G1374	5′-GCTCTGAGCACTGGAGAGAAAG-3′ 5′-RAGGAAAAYATGGAACCCAAAG-3′	Real-time PCR Northern
hCyclophilin	BC013915 (nt 161–685)	G1373 G1374	5′-GCTCTGAGCACTGGAGAGAAAG-3′ 5′-RAGGAAAAYATGGAACCCAAAG-3′	Northern Real-time PCR
hCOX2	L15326 (nt 20–1020)	G1206 G1208	5′-TCCTTCAGCTCCACAGCCAG-3′ 5′-CCCATTCAGGATGCTCCTGT-3′	Northern
mCOX2	M64291 (nt 52–1060)	G1212 G1213	5′-CACGAAGAATCTCAGCACTGC-3′ 5′-TCATCACCCCACTCAGGATGC-3′	Northern
hβ-Actin mβ-Actin	BC016045 (nt 449–713)	G513 G534	5′-TTAATGTCACGCACGATTTCC-3′ 5′-AGACCTTCAACACCCCAGCCA-3′	Northern

* For Northern blots, the PCR product was used.

prior to use. For ciglitazone (Biomol, Plymouth Meeting, PA) and lipopolysaccharide (LPS; Sigma) treatment, the PMA-activated U937 cells were pretreated with ciglitazone (20 μM) or vehicle (DMSO) for 12 h and then incubated in the presence of LPS (5 μg/ml) for the indicated time. The peripheral blood and bone marrow aspirate samples were from leukemia patients through an IRB-approved protocol. The diagnosis was verified by two independent hematopathologists using FAB classification based on the clinical, morphologic, cytochemical, and immunophenotypic data. The leukemic cells (>70%) of each specimen were isolated by centrifugation on Ficoll gradient, washed with PBS, and stored in RPMI with 30% FCS containing 10% DMSO in liquid nitrogen until use. 3T3-L1 adipocytes were grown in DMEM containing 25 mM glucose, 10% fetal bovine serum, and antibiotics. After inducing confluent preadipocytes for 2 days in culture medium supplemented with insulin (1 μg/ml), isobutylmethylxanthine (0.5 μM), and dexamethasone (1 μM), cells were maintained for additional 2 days in culture medium with insulin (1 μg/ml). At Day 9, more than 95% of cells exhibited the morphology of terminally differentiated adipocytes.

Total RNA was prepared with Trizol following the manufacturer's instructions (BD Bioscience). Fifteen micrograms of total RNA per lane was loaded for Northern blot analysis and blotted onto Nitron-Plus (Schleicher & Schuell, Keene, NH). cDNAs for mouse and human resistin and human leptin (CB265926) were random-labeled with [^{32}P]dCTP (Stratagene, La Jolla, CA). Hybridization was carried out at 65 °C in Rapid-hyb buffer (Amersham Bioscience, Piscataway, NJ) and blots were washed twice with 0.5× SSC/1% SDS at 65 °C (stringent wash) and visualized by PhosphorImager (Amersham Biosciences) or X-ray film.

PCR analysis. Reverse transcription was carried out in a reaction volume of 20 μl containing 1 μg of total RNA, polyT primer, and MMLV reverse transcriptase using the Advantage kit (BD Bioscience). Conventional PCR was performed under conditions typically consisting of 28 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s unless otherwise stated in the text. Quantitative real-time PCR was conducted with a Smart Cycler (Cepheid, Sunnyvale, CA) in 25 μl mixture containing 2.0 mM Mg^{2+}, 0.3× SYBR Green I (BioWhittaker Molecular Applications, Rockland, ME), 0.25 mM dNTPs, 0.5 μM of each primer, 1.5 U *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA), and 2 μl cDNA mixture of each sample. The real-time PCR conditions for resistin were denaturation at 96 °C for 4 min followed by 45 cycles of 94 °C for 45 s, 58 °C for 45 s, and 72 °C for 30 s. PCR conditions for cyclophilin A were 96 °C denaturation for 4 min followed by 45 cycles of 94 °C for 60 s, 59 °C for 60 s, and 72 °C for 45 s. The fluorescence cycle threshold (Ct) was calculated to quantitate the relative amount of gene expression. Cyclophilin A was used as a reference for initial RNA loading. Relative transcript levels was calculated as $x = 2^{-\Delta\Delta Ct}$, where x-fold is the difference in amount of the starting cDNA between 2 samples, $\Delta\Delta Ct = [(Ct1R - Ct1Cyc) - (Ct2R - Ct2Cyc)]$. Ct1R = average Ct of sample 1 for resistin, Ct1Cyc = average Ct of sample1 for cyclophilin A, Ct2R = average Ct of sample 2 for resistin, and Ct2Cyc = average Ct of sample 2 for cyclophilin A [14].

Comparative analysis of syntenic regions containing RELMs between the mouse and human. To localize and compare the mouse and human genomic sequences containing RELMs, we used BLAST (www.ncbi.nih.gov/BLAST/) for sequence similarity search of the mouse or human RELMs against nr and htgs databases. The syntenic chromosomal regions were determined by MapViewer (www.ncbi.nih.gov) and Mouse Synteny View (http://www.ensembl.org/Mus_musculus/syntenyview). Relevant genomic sequences were retrieved from GenBank and gene arrangement was referenced to the annotation of the contig. Where appropriate, some genomic sequences were further analyzed by (http://ccr-081.mit.edu/Genscan.html) or the Blast 2 Sequences (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html).

Phylogenetic tree. Amino acid sequences of RELMs were retrieved from GenBank for construction of the phylogenetic tree using the neighbor-joining method from a distance matrix based on mean amino acid difference (Genetics Computer Group, Madison, WI).

Results

Distinct tissue distribution of resistin between humans and mice

To compare the expression profiles of resistin between human and mice, we performed multi-tissue Northern blot analysis. As shown in Fig. 1, resistin is highly expressed in white adipose tissue in mice as well as in differentiated mouse 3T3-L1 adipocytes, but not in undifferentiated adipocytes. A light band of resistin was seen in mouse brown adipose tissue and muscle, probably due to contamination by white adipose tissue. In contrast, resistin gene expression was undetectable in human subcutaneous or omental adipose tissues by Northern blot analysis, and only a faint band at the size of resistin was observed in lung. As a reference, leptin expression was readily detectable and was more abundant in subcutaneous than in omental fat, which is consistent with previous publications [15,16]. Remarkably, resistin is highly expressed in the human U937 monocytic cell-line (also see Fig. 2).

To quantitatively determine the expression level of resistin, we conducted real-time PCR. Cyclophilin A was chosen as the RNA loading reference since its expression is ubiquitous and relatively consistent, in contrast to β-actin and GP3DH, whose levels change with adiposity and are not appropriate as references for comparison of gene expression between adipose and non-adipose tissues [17]. As shown in Fig. 1B and Table 2, at a similar expression level of expression of cyclophilin A in mouse white adipose tissue (Ct = 19.95 cycles), U937 cells (Ct = 19.63 cycles), and human fat tissue (Ct = 19.91 cycles), resistin mRNA was more abundant than cyclophilin in the mouse white adipose tissue in that it appeared earlier (Ct = 17.11 cycles). In contrast, the resistin expression curve from human adipose tissue appeared much later than the cyclophilin (Ct = 24.99 cycles). Resistin in human U937 cells appeared at Ct of 21.5, 3.3 cycles fewer than in the adipose tissue. After normalization for cyclophilin A, the level of human resistin gene expression in adipose tissue was estimated to be about 1/242 that of the mouse counterpart in adipose tissue and 1/8 that in human U937 cells.

Resistin is highly expressed in human leukemic cells

Since U937 is a cell line of human leukocyte origin with monoblastoid differentiation [18], we asked whether human leukemic cells express resistin and whether the expression of resistin is lineage-specific. Peripheral and bone marrow leukemic cells were examined for resistin expression. Leukocytes from both acute myelomonocytic leukemia (AML) and acute lymphoblastic leukemia (ALL) expressed resistin at a comparable level to U937, as did a human promyelocytic cell line, HL-60.

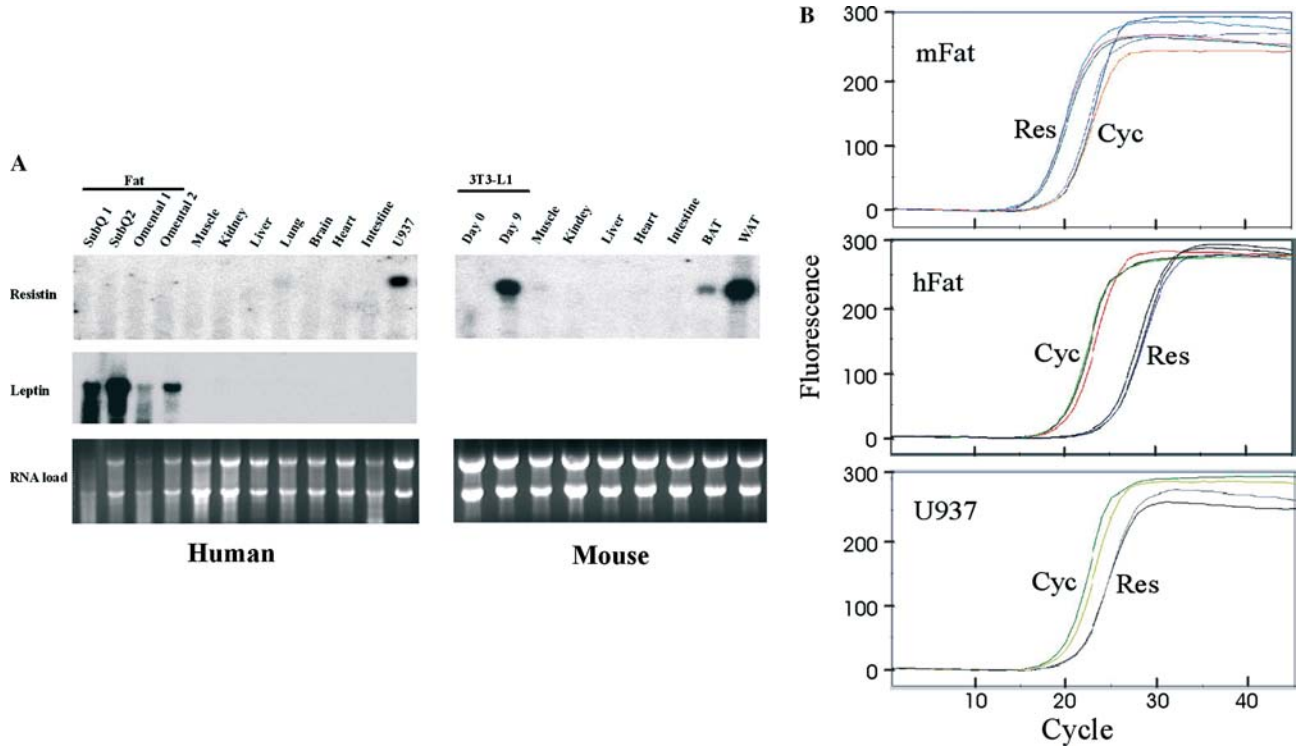

Fig. 1. Comparative analysis of resistin gene expression between humans and mice. (A) Tissue distribution of resistin. Blots containing total RNA (15 μg/lane) from indicated tissues including mouse 3T3-L1 preadipocytes (Day 0), differentiated adipocytes (Day 9), brown adipose tissue (BAT), white adipose tissue (WAT), human subcutaneous (subQ) or omental fat tissues from two different donors, and human monocytic cell line U937, which were probed with ^{32}P-labeled mouse or human resistin cDNAs. The human blot was stripped and reprobed with a human leptin cDNA probe. RNA loadings as determined by ethidium bromide staining of the gel are shown below. (B) Amplification plots of real-time PCR for resistin (Res) and cyclophilin (Cyc) in mouse adipose tissue (mFat), human adipose tissue (hFat), and human U937 cells. The Ct (threshold cycle) values are shown in Table 2.

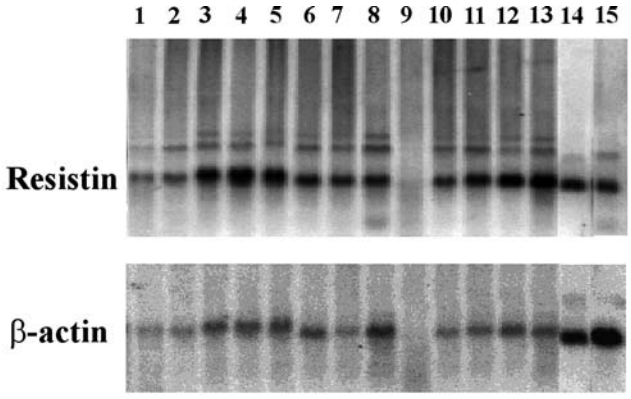

Fig. 2. Resistin expression in human leukemic cells. Northern blot containing 15 μg of total RNA was sequentially probed with human resistin and β-actin. Lanes 1–5 are from human peripheral blood cells, 6–13 from bone marrow cells. Lanes 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, and 13: acute myeloblastic leukemia from 11 different patients; lanes 5 and 12: acute lymphoblastic leukemia from 2 different patients. Lane 14 and 15 are human leukemia cell lines HL60 and U937, respectively.

No clear correlation was observed between the expression level and disease stage or cell source (peripheral or bone marrow). These results demonstrate that resistin is highly expressed in leukocytes of myeloblast and lymphoblast origin.

Table 2
Fluorescence cut threshold for each sample

	Cyclophilin A	Resistin
mWAT 1	20.07	16.94
mWAT 2	19.67	17.28
mWAT 3	20.12	17.12
U937-1	19.32	21.43
U937-2	19.94	21.57
hFat 1	19.79	25.16
hFat 2	20.30	25.15
hFat 3	19.65	24.67

Resistin expression in U937 and Raw264 cells is not regulated by LPS or ciglitazone

The high expression level of resistin in leukocytes raises the possibility that resistin may be involved in the inflammation process, and possibly insulin resistance also associated with inflammation. Hence, we examined whether resistin expression in human U937 and mouse Raw264 cell lines was regulated by LPS or the PPARγ

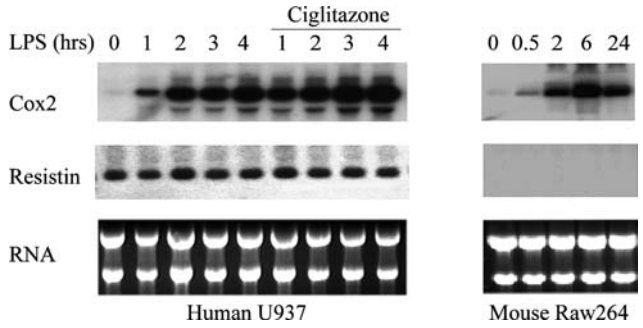

Fig. 3. Resistin is not regulated by ciglitazone or LPS. Human U937 cells were pretreated with or without ciglitazone (20 μM) 12 h before incubation with LPS (5 μg/ml) for indicated time points (left panel). Mouse RAW264 cells were only treated with LPS (5 μg/ml). The blots containing 15 μg of total RNAs from U937 or RAW264 cells were sequentially hybridized with human or mouse cyclooxygenase 2 (Cox 2) and resistin probes, respectively.

agonist ciglitazone, using cyclooxygenase 2 (Cox 2) as a marker of cell activation. LPS treatment induced Cox2 gene rapidly, as expected, but it did not induce resistin in either cell line (Fig. 3, and data not shown). Pretreatment with the PPARγ agonist ciglitazone increased Cox 2 gene expression about 2-fold but did not affect resistin expression in U937 cells. Notably, mouse Raw264 cells, which are considered to share many biological properties with human U937 cells, do not express resistin at all.

Human resistin is the true ortholog of murine resistin

The great difference in resistin gene expression between the human and mouse prompted us to examine whether human resistin is evolutionarily related to mouse resistin or simply similar in sequence. A phylogenetic tree was constructed using human and mouse RELM protein sequences. As depicted in Fig. 4A, human resistin is more related to mouse resistin than to mRELMβ and mRELMα, indicating human resistin is likely the true ortholog of mouse resistin. Since the possibility of the existence of additional RELMs in human that may be more evolutionally related to mouse resistin cannot be ruled out, we further mapped the chromosomal localizations of human and mouse resistin genes. The mouse and human resistin cDNA sequences were used as probes to search their corresponding high-throughput genomic sequences (htgs) or genome sequence databases for their chromosomal localizations. The human resistin gene *rstn* was localized to chromosome 19q13.3 in the NT_077812 contig, whereas the mouse *rstn* at chromosome 8A1 in the NT_039455 contig. The genes flanking the resistin gene, such as STXBP2 and FCER2, share significant homology between the two species and are in the same order, indicating these two regions are syntenic or mutually orthologous (Fig. 4B). Hence, we conclude that human *rstn* is the ortholog of mouse *rstn*.

Only two RELM mRNAs are expressed in humans

A combinational approach was taken to address the intriguing question of whether there is an additional RELM in humans that has yet-to-be identified. BLAST searches, coupled with MapView (www.ncbi.nih.gov/mapview) and ContigView (www.ensembl.org/Mus_musculus/contigview), revealed that murine RELMα and RELMβ are clustered within a 25-kb region on chromosome 16B5 (~48.8 Mb) in mice and contained in the NT_039624 contig from 45912673 to 45914343 nt (RELMα) and 45886926 to 45888469 nt (RELMβ). Human RELMβ resides on chromosome 3q13.1 in the NT_022434 contig (1737794 to 1740111 nt). Scrutiny of this contig using human and mouse resistin peptide

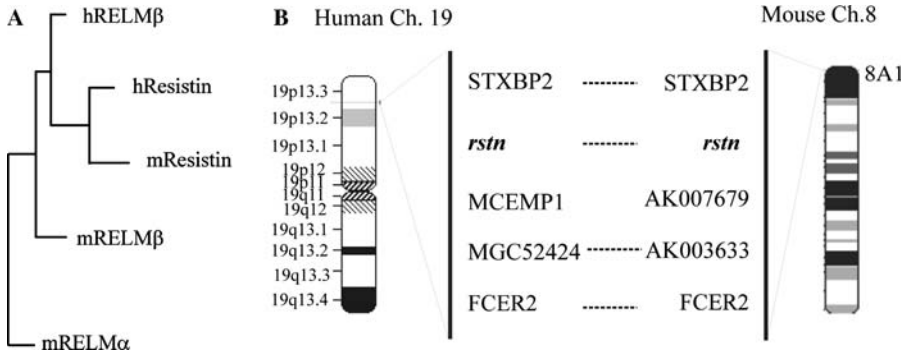

Fig. 4. Human resistin is the otholog of mouse resistin. (A) Phylogenetic tree for the human and mouse RELM sequences. The sequences were aligned using the CLUSTAL method and the tree was constructed using the neighbor-joining method with human (h) resistin (GenBank NP_065148), hRELMβ (NP_115968), mouse (m) resistin (NP_07360), mRELMα (NP_065255) and mRELMβ (NP_076370). (B) Gene arrangement at resistin loci in syntenic regions of human chromosome 19 and mouse chromosome 8. The human resistin gene *rstn* (bold) is located in a region of chromosome 19q13.3 syntenic to the region of mouse chromosome 8A1 where the mouse resistin gene *rstn* (bold) resides. The genes that encode proteins with 60% or more homology between the human and mouse are linked with dashed lines (STXBP2: syntaxin binding protein 2, NP_035633; MCEMP1: mast cell-expressed membrane protein 1 variant, NP_777578; MGC52424: XP_058961; and FCER2: low affinity IgE Fc receptors, NP_001993).

sequences disclosed another sequence homologous to RELMs (Fig. 5A), about 30-kb downstream from the human RELMβ gene. Significant conservation was observed between the predicted amino acid sequence of the putative RELM with that of the RELM family in the RELM signature region (Fig. 5B). Therefore, this sequence is likely to be the missing RELMα in humans. To determine whether this sequence is likely to encode an expressed gene, we scanned this region with Genscan, a bioinformatics tool designed to discover or predict genes from genomic sequence based on cognate splicing sites. Genscan predicted hRELMβ along with other genes in this region but failed to disclose any gene that would contain the putative RELM sequence (data not shown), suggesting that its adjacent sequence structure does not contain conserved splicing sites. We searched the putative RELM sequence against the expressed sequence tag (EST) database and found no perfect matches. By contrast, human resistin and RELMβ yielded 11 and 9 perfect hits, respectively, indicating there is no EST correspond-

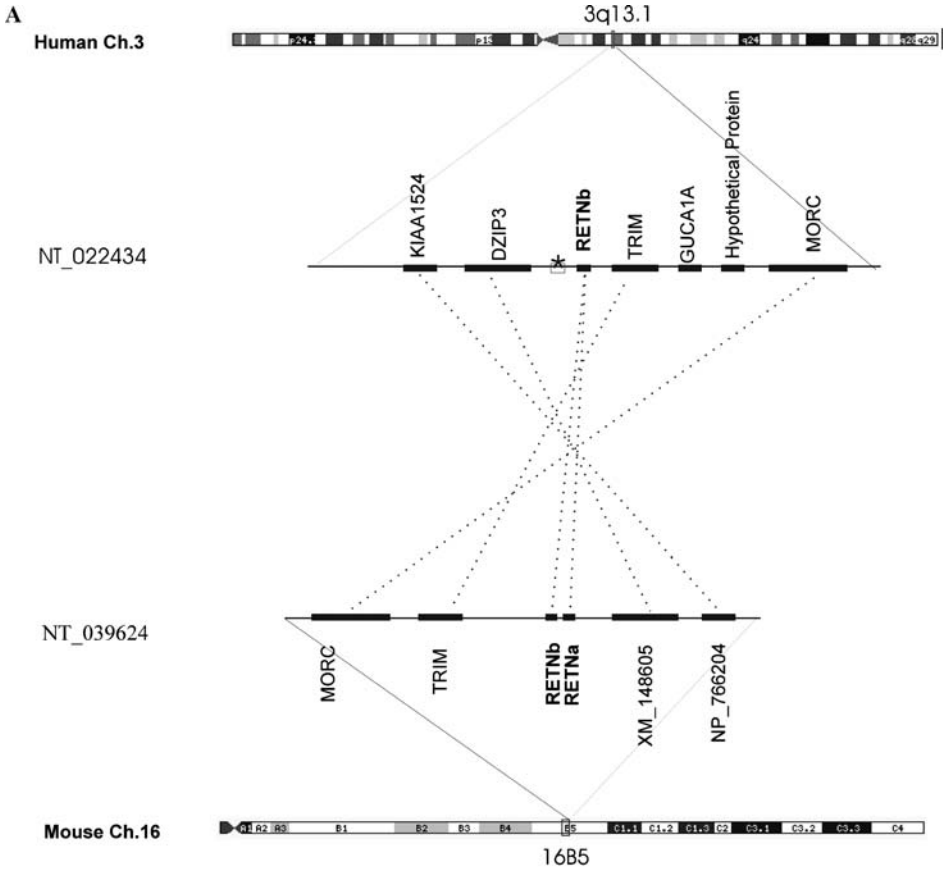

Fig. 5. Comparative analysis of RELMα and RELMβ between the human and mouse. (A) Schematic comparison of gene arrangement along the syntenic region between the human and the mouse. Human (h) RELMβ (RETNb, bold) is on chromosome 3q13.1 on the NT_022434 contig whereas mouse RELMα and RELMβ (RETNa and RENTb, bold) are on chromosome 16b5 on the NT_039624 contig. The transcribed or predicted genes are aligned with reference to annotation of the contigs and marked with black boxes. The blank box below the star indicates a sequence that is homologous to RELM but is not expressed as determined by EST and Genscan analysis. The genes with significant conservation (>50% identity) between the human and mouse are connected by dotted lines. The sizes of genes and intergenic distances are illustrative and not to scale. (B) Comparison of the putative RELM region with those of other RELMs in humans. The resistin peptide sequence was searched with tBLASTn against the human and mouse genomic contigs containing RELMα or RELMβ. The previously unknown RELM-homologous sequence (RELM-homo seq) is aligned with reported RELMs in the human and mouse. Black shading indicates amino acid identity of four or more family members, compiled from BLAST search results. The number indicates the positions of resistin peptides (human: NP_065148; mouse: AAM46862) and their corresponding nucleotides at the genomic contigs (human: NT_022434; mouse: NT_039624).

ing to the putative RELM. Hence, both Genscan and EST searches do not support expression of this putative RELM sequence. We thus conclude that only two RELMs, resistin and hRELMβ, exist in humans.

Discussion

Whether resistin is a link between obesity and insulin resistance, particularly in humans, is still a debatable question. To examine the possible role of resistin in the pathogenesis of obesity and insulin resistance in humans, we conducted this comparative study to determine the differences and similarities of resistin with respect to gene expression, regulation, and genomics between the human and mouse. Although low resistin expression in human adipose tissue has been reported by several studies using PCR, we demonstrated extensively that the level of resistin gene expression in human adipose tissue is undetectable by Northern blot analysis and is about 1/250th of the level in mouse adipose tissue.

Since adipocytes and granulocytes are under similar regulatory control during differentiation [19], we speculated that the resistin gene might be expressed in leukocytes. We thus examined the human cell lines U937 and HL60, both of myeloid origin, for resistin expression. Indeed, resistin gene expression was readily detectable by Northern blot analysis. Further study disclosed that human primary leukemia cells express this gene. Since resistin is highly expressed in both the leukemia cells and cell lines U937 and HL60, all of which are immature hematopoietic elements from both myeloid and lymphoid origin, resistin may play a role in the early stage of differentiation of those hematopoietic precursors in bone marrow.

Considering that RELMα/FIZZ1, a resistin homolog, is induced by inflammatory stimuli in type II pneumocytes [11], we hypothesized that resistin might be regulated like a cytokine in human monocytic cells. Human U937 and mouse RAW264 cells respond to LPS challenge by rapid induction of inflammatory genes such as cyclooxygenase-2 (Cox 2), TNFα, and nitric oxide (NO) synthase [20–23] and are typical models for functional or regulatory studies of cytokines [24–26]. However, the level of resistin expression in U937 is neither stimulated by LPS nor regulated by ciglitazone, indicating that resistin behaves differently from a typical inflammatory mediator in these cells.

Both suppression and induction of Cox 2 gene expression by PPARγ agonists have been reported [27–30], probably dependent on the cell type and ligand being tested. We did not detect any noticeable regulation of resistin by ciglitazone (20 μM, 12 h) in U937 cells, which is not in agreement with a recent report in which rosiglitazone (100 nM, 96 h) suppressed resistin gene expression in bone marrow cells [31]. The difference in cell type, agonist, or treatment time likely contributes to the disparity. Notably, resistin is not expressed in the mouse monocytic cell line RAW264, which shares many biological properties with those of human U937 [24–26], another demonstration of the difference in resistin gene expression between the human and mouse.

Thus, there is a dramatic difference in resistin gene expression between the mouse and human. The resistin gene is highly expressed in adipocytes in mice but not in humans. By contrast, leukocytes are the major site of expression for resistin in humans. The mechanism for the difference is not clear. C/EBPα is a transcription factor which has been demonstrated recently to be critical for optimal resistin expression by binding to a C/EBP site in the mouse resistin gene promoter [32]. This factor is highly expressed in human adipocytes and is critical for adipogenesis [33,34] and is therefore unlikely to be the reason for the low expression of this gene in human adipose tissue. Conversely, sequence divergence between the promoter regions of resistin in the two species may contribute to the difference in expression. Indeed, our analysis of 1 kb of the proximal promoter region of the resistin gene found no significant conservation between human and mouse (data not shown).

Human resistin was named after its mouse counterpart based on a protein similarity of only 53% identity. The low conservation at the protein level and high divergence in its expression between the two species [10], in addition to the fact that only two RELMs have been discovered in humans, led us to question whether human resistin is the true ortholog of mouse resistin. Phylogenomics is an approach that combines genome sequence information and phylogenetic studies into a composite analysis [35,36], which improves functional predictions for uncharacterized genes. Our study revealed that the chromosomal localizations of human and mouse resistin genes are syntenic. In addition, a phylogenetic tree indicates that human resistin is closest to the mouse counterpart in evolution. Therefore, human resistin is the ortholog of mouse resistin. However, it is still questionable whether the orthologs play similar biological roles, and in particular, whether resistin serves as link between obesity and insulin resistance in humans, considering the significant divergence in sequence similarity and tissue distribution. The dramatic difference in gene expression preludes a degree of functional speciation of this gene during evolution. In other words, resistin may play different biological roles in different species. Nevertheless, this study does not exclude the possibility that human resistin released from leukocytes may have insulin-resistant activity or that it may have other yet-to-be discovered conserved biological functions.

The fact that only two RELMs have been discovered in humans compared to three in mice caused us to

speculate that there might exist an additional yet-to-be-discovered human RELM that may be closer to mouse resistin with respect to function and expression. We have discovered a RELM-homology region in the vicinity of human RELMβ and determined from bioinformatics analysis that this sequence is not likely to be expressed, providing a likely explanation for why there are only two RELMs in human.

In summary, a striking difference exists in gene expression between human and mouse resistin genes despite their orthologous relationship. In addition, the divergence of the RELM family in sequence and in member number between the two species suggests a possible speciation in function during evolution. High expression of the resistin gene in leukemic cells in humans is an interesting observation and is worthy of further investigation.

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