Alternative splicing generates a novel non-secretable resistin isoform in Wistar rats[1]

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Abstract **Resistin is a secreted adipose tissue hormone that belongs to the resistin-like molecule family. We report here a new alternatively spliced isoform of the rat resistin gene, named S-resistin (short resistin), detected in adipose tissue by reverse transcription-polymerase chain reaction (RT-PCR). A comparison of this cDNA variant and genomic sequences indicates the lack of the second exon containing the secretory consensus signal. Both cDNAs, resistin and S-resistin, were carboxy-tagged with FLAG epitope and transiently expressed in cultured cell lines. While the resistin-FLAG construct gives the expected pattern for a secretion protein, the S-resistin-FLAG construct yielded a predominant nuclear staining. These results indicate that this splicing event regulates the fate and probably the function of the mature protein.**

Key words: Resistin; Resistin-like molecule; Short resistin; Adipogenesis; Adipose tissue

1. Introduction

Resistin is a novel adipose-secreted hormone induced during the late stage of adipogenesis and constitutively expressed in white adipose tissue (WAT) [1–4]. The inhibitory effect of resistin on adipocyte differentiation suggests that it acts as a feedback regulator of adipogenesis [5]. Resistin mRNA encodes a 114 amino acid polypeptide including a 20 amino acid signal peptide and a carboxy-terminal cysteine-rich domain characteristic of the resistin-like molecule (RELM) family. Other members of this family, RELMα and RELMβ, are also secreted proteins with tissue-specific distribution. Thus, RELMα is expressed in lung, tongue and stroma vascular fraction of adipose tissue, and RELMβ in epithelial intestine cells [6–9].

Resistin serum concentration is increased in diet-induced obese mice and decreased by anti-diabetic drugs [1]. Moreover, in vivo administration of recombinant resistin impairs glucose tolerance and insulin action in normal mice, while administration of anti-resistin antibody enhances the insulin sensitivity of insulin-resistant and obese mice. These facts allowed Steppan et al. [1] to propose that resistin is a hormone that links obesity to insulin resistance and type II diabetes. However, several studies have been published supporting the concept that insulin resistance and obesity are associated with decreased resistin expression [10–12]. Since the resistin receptor has not yet been isolated and the molecular mechanism of resistin action still has not been elucidated, its role remains unclear.

In the process of analyzing the expression of resistin in adipose tissues from Wistar rats, we found a shorter resistin cDNA, S-resistin, generated by alternative splicing. This splicing variant eliminates the second exon containing the secretion peptide signal, and could produce a non-secretable protein. Interestingly, S-resistin is found into nucleus of transfected cell lines, opening the possibility, not reported previously, that resistin could also play a role as an intracrine factor in the regulation of adipocyte gene expression and/or in the adipogenic process.

2. Materials and methods

2.1. Cell culture

Stroma vascular preadipocytes were isolated under sterile conditions from epididymal fat pads of 180–220 g male Wistar rats fed ad libitum. The fat pads were digested with collagenase according to the method of Rodbell [13]. The preadipocytes were obtained and differentiated as previously described [14]. Cells were regarded as differentiated by morphologic criteria assessed by oil red O staining. RNA was extracted from preadipocytes and differentiated adipocytes as described below.

2.2. RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA extraction from 3 months old male Wistar rat adipose tissues and cells was performed as described by Peralta et al. [15] and Carraro et al. [16] respectively. cDNA synthesis and PCR reactions were performed as described previously [15]. The primers used for full-length resistin cDNA PCR amplification were primer 1 (5′-AGTTGTGCCCTGCTGAGCTCTCTGCC-3′) as forward, and primer 4 (5′-CCCATTGTGTATTTCCAGACCCTC-3′) as reverse one. Both were designed according to the published sequence [5] (accession num-

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Abbreviations: RELM, resistin-like molecule; S-resistin, short resistin; FBS, fetal bovine serum

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ber in the TIGR database: TC119941) and resulted in the amplification of a 529 bp fragment. Additional primers were used for cDNA characterization: primer 2 (5′-CACTGTGTCCCATGGATGAAGCC-3′), primer 3 (5′-CCCTCTGGAGACTGACCAGC-3′), and primer 5 (5′-TGAGTCCACTTTACCGTGCATC-3′). Moreover, we designed the primer X (5′-TGAGCTCTCTGCCACGTGCC-3′) as a forward hybrid primer that contains the 16 last nucleotides from the first exon and the four first nucleotides from the third exon. The rat actin primers used were as described previously [14]. The PCR reactions under standard conditions were performed at 1 μM primer concentration. To amplify resistin cDNAs under non-competitive conditions, the resistin primer concentration in the PCR reaction was three-fold the standard one. Following reactions, samples were electrophoresed in 2% agarose gels, and the amplified bands were analyzed as described by Peralta et al. [15]. Control reactions with different number of cycles have been previously performed to select conditions of amplification in the linearity range.

2.3. Construction of plasmids

Amplified PCR products using primers 1 and 4 were cloned into pSTBlue-1 plasmid using blunt cloning kit (Novagen) and sequenced with the ABI Prism 3700 DNA analyzer (Applied Biosystems). An eight amino acid FLAG epitope tag (peptide DYKDDDDK) was introduced at the carboxy-terminus immediately preceding the termination codon as described previously [17]. The carboxy FLAG-tagged products obtained, S-res-FLAG and res-FLAG, were subcloned into

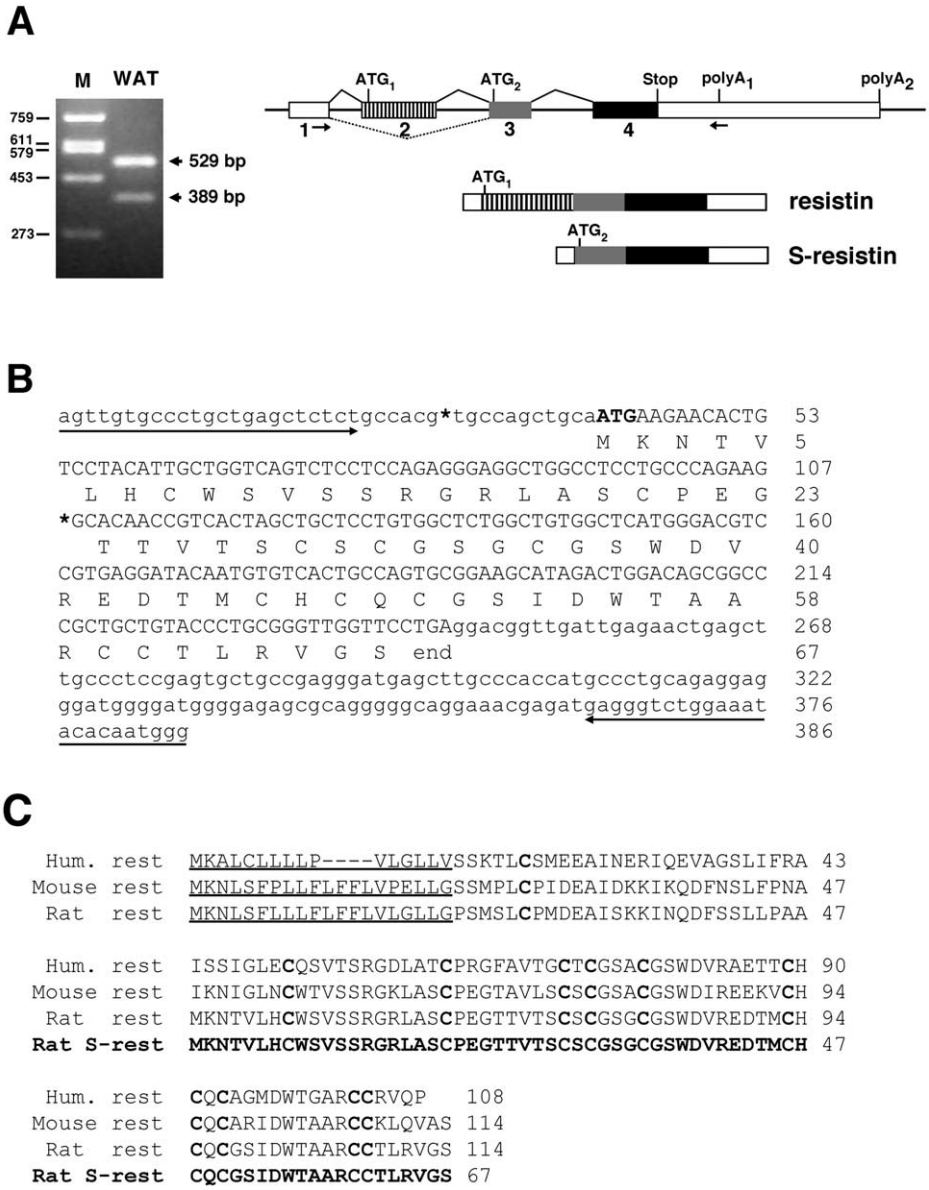

Fig. 1. Identification of a novel alternatively spliced rat resistin. A: RT-PCR amplification of rat resistin mRNAs from WAT using primers 1 and 4 (see Section 2). The 529 bp cDNA corresponds to the resistin variant reported previously [5]; the 389 bp cDNA was identified as a novel spliced resistin variant. At the right side, a schematic picture of rat resistin genomic structure and its putative transcripts is shown. White boxes represent the 5′ and 3′ untranslated regions, the second alternative exon is indicated as a dashed box, and the third and fourth exons as gray and black boxes respectively. Arrows show the positions of PCR primers 1 and 4 used. The two potential translation start AUG sites are indicated. Alternative splicing event is denoted by a dashed line. B: S-resistin cDNA sequence. Coding sequences are capitalized. Initial ATG is shown in bold. The position of primers used is indicated by arrow. The junction points of the splicing process are marked by asterisks (accession number AJ555618). C: Multiple sequence alignment of resistin proteins. Alignment between rat, human and mouse resistin (accession numbers NP653342, NP065148 and NP075360, respectively), and S-resistin variant was performed by ClustalW program (EMBL-EBI, European Bioinformatics Institute). The endoplasmic reticulum targeting peptides of large isoforms are underlined. Conserved cysteine residues are indicated in bold.

pCMV5 expression vector to finally obtain pCMV5-S-res-FLAG and pCMV5-res-FLAG.

2.4. Cell line culture and transfection

COS-7 and HEK-293T cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS) (Gibco BRL) at 37°C in a 7% CO_2 atmosphere. 3T3-L1 cells (purchased to ATCC) were grown in DMEM complemented with 10% FBS. Cells were transiently transfected using lipofectamine™ reagent (Invitrogen). Cells were incubated with DNA–liposome complexes in serum-free media for 6 h. After 48 h, cells were fixed and processed.

2.5. Western blot analysis

Total protein extracts were obtained from transiently transfected HEK-293T cells. 10^6 cells were transfected with 20 μg of each DNA, and after 36 h were harvested in lysis buffer (1% Triton X-100, 150 mM NaCl, 20 mM Tris–HCl pH 7.5, 0.5 mM ethylenediamine tetraacetic acid (EDTA), 1 mM ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and protease inhibitors), incubated at 4°C for 15 min and centrifuged a $12\,000\times g$ to pellet the cell debris. Proteins were separated on 15% tricine–polyacrylamide gel electrophoresis (PAGE) gels and transferred to 0.2 μm nitrocellulose membranes. Blots were probed with anti-FLAG M2 antibody (1:2000, Sigma), washed and incubated for 30 min with horseradish peroxidase-conjugated anti-mouse antibody (1:1000, Bio-Rad). After washing, antigen–antibody complexes were detected using the enhanced chemoluminescence (ECL) method (Amersham Pharmacia Biotech).

2.6. Immunofluorescence analysis and mitochondria-specific staining

For the study of mitochondrial location, living cells were stained with MitoTracker Red CMXRos (200 nM, 30 min, Molecular Probes) and fixed. Immunofluorescence analysis with anti-Flag M2 antibody was performed as described previously [17]. Nuclear staining with Hoechst was performed by incubation with 1 μg/ml in phosphate-buffered saline (PBS) at 30 min. Fluorescence microscopy was performed using an axiovert epifluorescence microscope (Carl Zeiss) at ×100.

3. Results

3.1. Characterization of a novel alternatively spliced variant of rat resistin gene

During the analysis of resistin expression by RT-PCR in adipose tissues from young Wistar rats, we have detected the amplification of two different resistin transcripts, the expected resistin messenger and a shorter PCR fragment not described previously (Fig. 1A). Since PCR reactions were carried out using specific primers complementary to sequences upstream to the 5′ translation start site and 3′ untranslated region of rat resistin cDNA (primers 1 and 4, see Section 2) we thought that a process of alternative splicing could account for the generation of this novel fragment. To check this hypothesis, both PCR products were cloned and sequenced confirming that the major one, of 529 bp, contains the full-length resistin cDNA described by Kim et al. [5], whereas the minor PCR fragment, of 389 bp, presents identical sequences at 3′ and 5′ ends as rat resistin cDNA, but it contains an internal deletion of 140 nucleotides at its 5′ region (GenBank accession number AJ555618). This structure strongly suggests that this cDNA molecule represents a new spliced variant. Alignment analysis using BLAST program (NCBI) with rat genomic (chromosome 12 supercontig NW_042768) and cDNA resistin sequences indicates that flanking nucleotides of divergence point among sequences of this 389 bp fragment and resistin cDNA perfectly match with exonic sequences from exon 1/intron 1 and intron 2/exon 3 junctions, confirming that entire exon 2 is spliced in the shorter fragment. Fig. 1A shows the genomic structure of rat resistin gene and the splicing events involved in the generation of both resistin transcripts. Thus, the 389 bp cDNA contains the first non-coding resistin exon, but lacks the second one containing the AUG start translation codon which encodes for the 47 first amino acids of resistin protein including the 20 amino acid peptide signal sequence characteristic of secreted proteins (Fig. 1B, C). We have named this novel resistin isoform S-resistin.

A complete characterization of resistin variants performed with specific primers for each isoform is shown in Fig. 2. To amplify specifically the S-resistin variant, we realize RT-PCR analysis using a S-resistin-specific forward primer derived from exon 1/exon 3 junction (primer X, see Section 2, Fig. 2A and B). As is observed in Fig. 2B (lanes X-4 and X-5), with S-resistin-specific primer a single PCR fragment corresponding to S-resistin variant is detected. Also, the longer resistin isoform is specifically obtained with primer derived from exon 2 sequences spliced in S-resistin (Fig. 2B, lanes

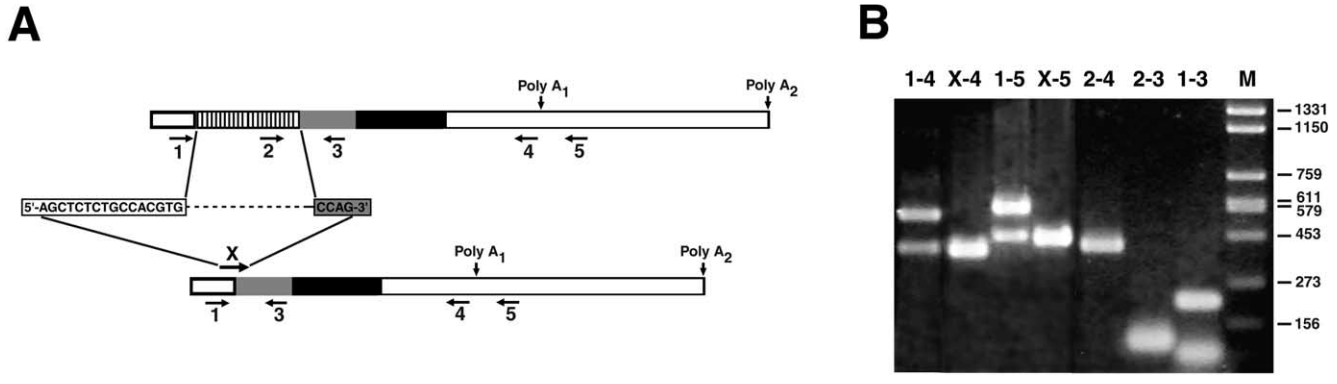

Fig. 2. RT-PCR characterization of resistin cDNA variants. A: Diagram of resistin isoforms with the relative position of the different primers used in RT-PCR assays. Numbered primers, one to five, are represented by arrows. Primer X sequence is indicated, the sequence derived from the exon 1 3′ end is shown in a white box and the sequence from the exon 3 5′ sequence in a gray box. B: RT-PCR analysis from WAT RNA. RT is made with random primers and equivalent aliquots of cDNA are used for PCR amplification. Primer pairs used in each PCR reaction are indicated at the top. Molecular weight markers are *Hin*dIII-digested ϕ29. The fragment size is indicated in bp. Both resistin and S-resistin sequences are amplified using a common forward primer (primer 1, lanes 1-4, 1-5 and 1-3). A single fragment is obtained when specific primers for each isoform are utilized. Primer X for S-resistin variant (lanes X-4 and X-5) and primer 2, derived for the exon 2 sequence, for the long resistin isoform (lanes 2-4 and 2-3). Identical pattern is obtained with reverse specific primers for the alternative polyadenylated resistin species described by Kim et al. [5] (primers 4 and 5, lanes 1-4 and 1-5). It shows that resistin and S-resistin use both polyadenylation signals.

2-4 and 2-3). As a control of this approach, primer X was used as forward primer to amplify cDNA using the clones from S-resistin and resistin. Only PCR amplified cDNA cloned sequences from S-resistin gave the predicted amplification product (data not shown). Furthermore, when PCR reaction was made in RNA samples without RT no bands were detected.

The predicted S-resistin open reading frame (ORF) encodes for a 67 amino acid long protein that conserves the carboxy-terminal cysteine-rich domain present in the RELM family (Fig. 1C) [1,5,7]. Its first available translational start site is located at the beginning of the third exon (Fig. 1A), and it corresponds to the internal Met48 residue of resistin (Fig. 1C). Although rat, mice and human resistin genes show identical genomic structures and high degree of homology at amino acid level, this methionine residue is only present in rat resistin protein (Fig. 1C). The lost N-terminal portion displays a slightly lesser similarity with its mouse and human resistin homologs, and, interestingly, it comprises the signal peptide for secretion (Fig. 1C). Thus, the new resistin form could represent a non-secretable variant molecule generated by alternative splicing of the rat resistin gene.

3.2. *Analysis of the expression of resistin isoforms in adipose tissue and during adipogenesis*

RT-PCR from epididymal and retroperitoneal adipose tissue RNA obtained from different animals shows appreciable expression levels of the major resistin transcript using primers at the standard concentration. In contrast, the S-resistin isoform appears as a band of slight intensity, being undetectable in some samples (data not shown). In order to clarify whether this pattern is due to a possible competence for primer binding, likely due to relative levels of transcripts, we performed PCR under non-competitive conditions in presence of high primer concentrations (see Section 2). Fig. 3A shows that under these conditions the novel resistin transcript is observed in all samples from epididymal and retroperitoneal fat tissue, and it persists when restrictive PCR is performed (annealing temperature until 80°C) (data not shown).

Quantitative analysis of both amplified cDNAs under non-competitive conditions is shown in Fig. 3B. It reveals that the relative expression of both resistin isoforms is similar in epididymal and retroperitoneal adipose tissues in all analyzed individuals (Fig. 3A). The estimated relative proportion of resistin and S-resistin cDNAs indicates that the expression of resistin is 1.5-fold higher than that of S-resistin in both adipose tissues (Fig. 3B). Interestingly, S-resistin transcript is also present in brown adipose tissue (BAT) although in lesser amounts than in WAT (Fig. 3C).

In addition, we have analyzed whether S-resistin expression, like resistin, is regulated during adipogenesis. For that purpose, we performed in vitro differentiation of stroma vascular preadipocytes isolated from rat WAT. RT-PCR assays for resistin expression of RNA obtained from stroma vascular preadipocytes and in vitro differentiated adipocyte cells are shown in Fig. 3C. These data show that resistin and S-resistin expression levels are barely detectable in the stroma vascular fraction but they increase during in vitro adipogenesis. The levels of expression detected for both resistin isoforms in differentiated cells are similar to that of WAT (Fig. 3C). Thus, apparently, resistin and S-resistin expressions are induced during adipogenesis.

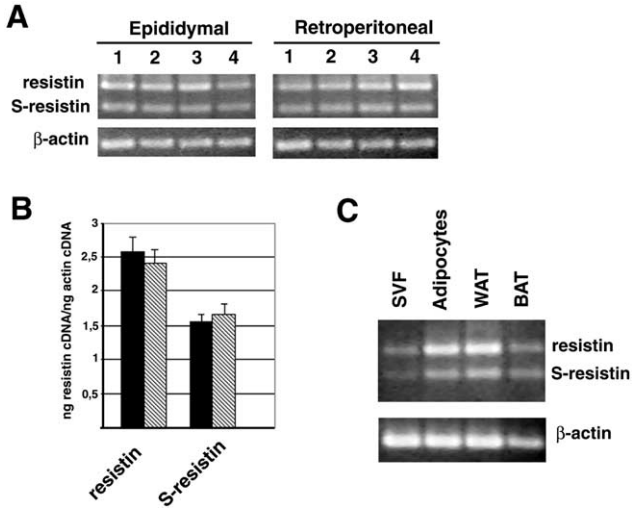

Fig. 3. Expression analysis of resistin isoforms in adipose tissues. A: Expression of resistin and S-resistin was determined by RT-PCR in both epididymal and retroperitoneal adipose tissues from four 3 months old rats, as indicated in Section 2. The amplification of a 356 bp fragment of β-actin is shown as control. B: Quantification analysis of amplified products in epididymal (black box) and retroperitoneal (dashed gray box) tissues, was performed in each sample as the ratio of ng of resistin or S-resistin cDNA amplified per ng cDNA of β-actin under the reaction conditions used. Values are means ± S.E.M. of four separate determinations per adipose tissue type. C: Stroma vascular fraction from fat pads was subjected to in vitro adipocyte differentiation. RT-PCR was performed from cells as well as from WAT and BAT RNAs. Actin amplification is shown as control.

3.3. *S-resistin is localized into the nucleus*

As S-resistin lacks secretion signal peptide we examined its 67 amino acid sequence searching for relevant signals for specific intracellular localization. Although we could not find any relevant signal for specific targeting, using a PSORT II program developed by Reinhardt and Hubbard [18], which discriminates the tendency of a protein to be at either the nucleus or the cytoplasm based on the amino acid composition, a nuclear localization of S-resistin was predicted.

To test this likely differential intracellular distribution between S-resistin and resistin, both alternatively spliced cDNAs were tagged with FLAG epitope at the carboxy-terminus and transiently expressed in COS-7, HEK-293T and 3T3-L1 cells (Fig. 4). Western blot analysis confirmed the size of expressed proteins. As shown in Fig. 4B, a unique band of expected mass is detected with anti-FLAG antibody M2 in total lysates obtained from HEK-293T cells transiently transfected with each, pCMV5-S-res-FLAG or pCMV5-res-FLAG, expression vector. For S-resistin-FLAG, a single product of about 7.5 kDa, according to its predicted ORF, is observed (Fig. 4B). In agreement with previous reports [1,5,7], in resistin-FLAG-transfected cells a unique peptide of about 10 kDa is found (Fig. 4B), indicating that when the start AUG codon is present, other downstream start codons are not used, in accordance with the scanning model of translation initiation. It is therefore necessary that the splicing event occurs for the translation of the S-resistin protein.

The precise intracellular distribution of resistin isoforms was analyzed by staining COS-7 cells, transiently transfected with pCMV5-S-res-FLAG or pCMV5-res-FLAG constructs, with anti-FLAG antibodies. Fig. 4C shows positive COS-7

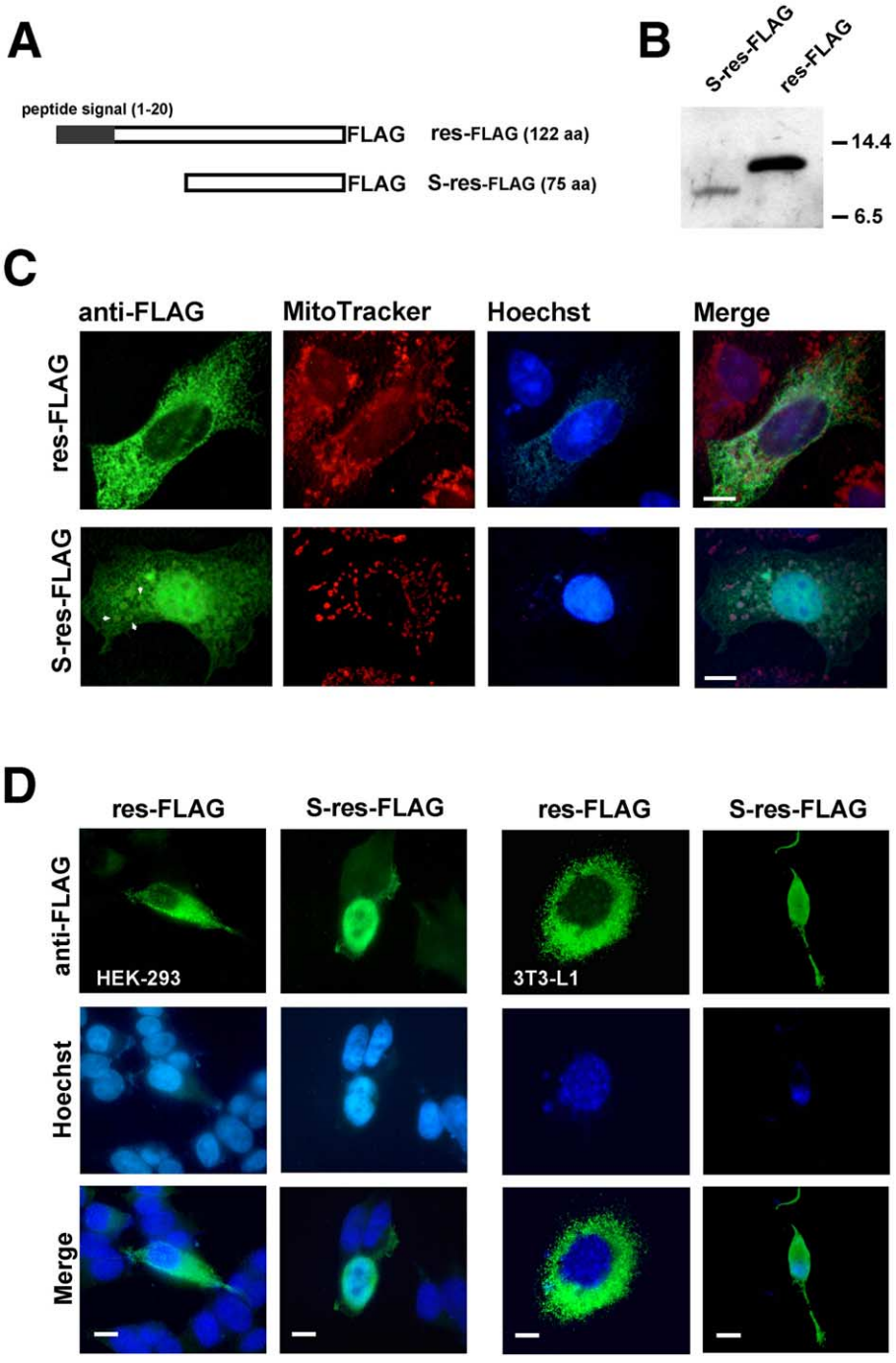

Fig. 4. Rat resistin isoforms show different intracellular distribution. A: Structure of rat FLAG C-terminus-tagged resistin isoforms. For res-FLAG the secretion signal is indicated. B: Western blot analysis for expressed resistin isoforms fused with FLAG epitope. HEK-293T cells were harvested 48 h after the transfection, solubilized, and extracts (20 μg of protein) were analyzed by immunoblotting with M2 anti-FLAG antibody as described in Section 2 (1:2500 dilution). Values beside the lanes are molecular size markers in kDa. C and D: Subcellular localization of Flag-tagged resistin isoforms in COS-7, HEK-293T and 3T3-L1 cells. Cells were transiently transfected with pCMV5-S-res-FLAG and pCMV5-res-FLAG vectors, and direct immunofluorescence was conducted with M2 anti-FLAG antibody. Anti-mouse fluorescein isothiocyanate (FITC) was used as secondary antibody. C: Transfected COS-7 cells were co-stained with anti-FLAG (green panels), MitoTracker (red panels) and Hoechst (blue panels). Also composed images are shown. Distribution of both resistin forms is clearly different, being predominantly nuclear for S-res-FLAG and cytoplasmic for res-FLAG. As shown, the resistin-FLAG pattern is not coincident with mitochondrial specific MitoTracker staining or nuclear signals. In contrast, using the S-res-FLAG construct, the most intense staining is detected into nucleus. Representative cells for each construct are shown. D: Transfected HEK-293T and 3T3-L1 cells co-stained with anti-FLAG and Hoechst. As shown, using the S-res-FLAG construct, intense staining is detected into nucleus although a remarkable cytosolic staining is also detected in both cell lines. At the contrary, the resistin-FLAG staining is exclusively cytosolic. In C and D scale bar is 20 μm.

cells for both resistin isoforms confirming their differential localization. For res-FLAG the expected pattern for a secretion protein is obtained, with positive cells displaying a punctuate cytoplasmic staining with intense signals in endoplasmic reticulum and trans-Golgi network (Fig. 4C). No signal is observed into the nucleus or into the cytosol. In sharp contrast, S-res-FLAG construct yields a quite different distribution. Although cytoplasmic staining can also be observed to some extent, S-res-FLAG isoform is clearly enriched in the nuclei of COS-7 cells (Fig. 4C). This cytoplasmic staining does not resemble to secretion pattern of resistin and is not coincident with signals of mitochondrial markers. Double staining with MitoTracker (a mitochondrial specific fluorescence dye) and anti-FLAG antibody reveals a different pattern (Fig. 4C). Only in some cells cytoplasmic anti-FLAG-positive structures around MitoTracker-stained organelles are observed (indicated by arrows in Fig. 4C). Nuclear staining is homogeneous and the fluorescence does not penetrate into the nucleolus. Labeling with Hoechst, which stained AT-rich DNA, demonstrates a co-localization of nuclear S-res-FLAG and DNA pattern (Fig. 4C, D). Similar results have been obtained using HEK-293T and 3T3-L1 cells, confirming that this subcellular targeting is not restricted to COS-7 cells (Fig. 4D). In HEK-293T and 3T3-L1 cells we observe a more pronounced staining of cytoplasmic structures perhaps due to higher pCMV5-S-res-FLAG expression in both cell lines than in COS-7 cells. In accordance with that, immunofluorescence analysis performed 24 h after transiently transfection shows lower cytosolic signals than in cells analyzed after 48 h of transfection (data not shown).

4. Discussion

Here we describe a novel shorter cDNA variant molecule of rat resistin, that we have named S-resistin, generated by alternative splicing. Several authors have performed either RT-PCR or Northern resistin analysis from rat adipose tissues, but this S-resistin cDNA has not been described previously [5,11,19,20]. It is possible that the S-resistin variant has not been observed earlier due to its relative lower expression level with respect to the resistin isoform. In fact, in our hands clear S-resistin cDNA amplification by RT-PCR was only observed when reactions were made under non-competitive conditions, rising three-fold the primer concentration. Kim et al., using Northern analysis, have reported the presence of two resistin mRNA species of 1.4 and 0.8 kb in rat adipose tissues. These were not produced by splicing but by different election of polyadenylation site [5]. We detected that both, resistin and S-resistin, use both polyadenylation signals. Therefore, it is possible that the difference between S-resistin and resistin length is not enough to be detected by Northern analysis. Here we report the amplification of S-resistin cDNA in WAT, epididymal and retroperitoneal adipose tissues, and BAT, suggesting that this isoform is functionally relevant for both fat cell types.

The splicing event described herein eliminates the target sequence for the secretion pathway, contained in the spliced second exon, resulting in a new ORF that could generate a shortened non-secretable isoform of 67 amino acids. Although this minor isoform of rat resistin has not been detected in adipose tissues in vivo, the isolation of this shortened cDNA variant suggests that it could be functionally relevant in adipose cells. Thus, in contrast to results obtained in primary culture of human preadipocytes and adipocytes [12], we found that, as described for resistin in mice and rat [1,5], S-resistin expression is induced during in vitro adipocyte differentiation, suggesting that this new isoform could play an important role in adipogenesis.

Interestingly, when S-resistin cDNA is overexpressed in cell lines, a completely different cellular distribution from that of resistin is observed, with S-res-FLAG-transfected cells showing a predominant nuclear staining. Although sequence analysis predicted a preferential nuclear versus cytoplasmic localization, this nuclear pattern was unexpected because the S-resistin sequence lacks a defined nuclear localization signal. Nevertheless, this S-resistin nuclear distribution could be due to its low molecular weight. Molecules of less than 30–40 kDa can pass passively through the nuclear pore complex [21]. If diffusion was followed by intranuclear binding it would result in nuclear accumulation [22]. According to that, S-resistin nuclear accumulation seems to be a saturable process, so that when S-resistin-transfected cells show higher protein levels, increased cytoplasmic S-resistin signals are also observed.

Although we do not know yet the significance of nuclear localization for resistin function, several evidences have been developed over recent years indicating that some peptide hormones and growth factors can function in the intracellular space after internalization or directed retention within the cells where they were synthesized [23]. Similarly to resistin, for some growth factors alternative forms with nuclear localization have been described. The most widely characterized secreted proteins located into the nucleus are fibroblastic growth factor 2 (FGF-2) and parathyroid hormone-related peptide (PTHrP) whose nuclear forms are translated from in-frame non-AUG start sites [24,25]. Another example is ES-kine/CCL27, a novel β-chemokine that is differentially spliced to produce secretable and nuclear targeted isoforms [26]. The alternative nuclear isoform lacks the signal peptide and is translocated to the nucleus and when overexpressed in 3T3 cells causes cytoskeletal rearrangements [27].

No equivalent alternative spliced isoform to S-resistin has been described in other species. It is possible that in other mammals, like humans and mice, alternative mechanisms such as presence of non-AUG start sites, post-transductional processing or internalization of both ligand and active receptor into the cell nucleus, would account for generating a nuclear targeted resistin [23,25].

On the other hand, all members of the RELM family present at the C-terminal region a cysteine-rich domain containing 10 conserved cysteine residues that are likely involved in intramolecular disulfide bonds that define the conserved structure of RELMs. Another cysteine residue, Cys26, present in resistin and RELMβ, but not in RELMα, has been described as necessary for homodimerization [28,29]. It has been suggested that, like other hormone factors, RELM oligomerization is necessary for ligand-induced receptor activation [30–32]. In S-resistin, the alternative splicing eliminates this Cys26 conserving the C-terminal cysteine domain. Since S-resistin seems to be a non-secretable protein, it is possible that oligomerization is not necessary for its physiological function and, therefore, S-resistin could act into the nucleus as a monomeric molecule. Moreover, as described by Chen et al. [29], the RELM family members can also interact with each other regardless of the presence of Cys26 through non-

covalent interactions. Since both resistin and S-resistin are adipose-specific molecules, this raises the possibility of interactions between these two related RELMs.

Although the molecular identity of RELM receptors remains unknown, resistin as well as RELMα and RELMβ have been described as circulating hormones with a paracrine and autocrine function on adipose tissue. The existence of a non-secretable resistin isoform opens the possibility that intracellular resistin could exert at least part of its biological effects via intracrine actions at the level of the nucleus. However, further studies are required to understand the function of this protein.

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