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# Molecular cloning of cDNA encoding adipogenesis inhibitory factor and identity with interleukin-11

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A cDNA encoding a novel adipogenesis inhibitory factor (AGIF) that inhibits the process of adipogenesis in mouse 3T3-L1 preadipocytes was cloned from a cDNA library of the human bone marrow-derived stromal cell line KM-102. The cloned cDNA contains an open reading frame coding for an AGIF precursor of 199 amino acids. Analysis of the sequence of this cDNA revealed identity of this factor with a recently reported novel cytokine, designated interleukin-11 (IL-11). AGIF/IL-11 may play an important role in stromal cell-associated hematopoiesis through its regulatory action on adipocyte differentiation in the bone marrow microenvironment.

Adipogenesis inhibitory factor; Interleukin-11; cDNA cloning; Bone marrow stromal cell

## 1. INTRODUCTION

Long-term bone marrow cultures provide an in vitro system in which hematopoiesis can be maintained for several months. Such cultures consist of a mixture of preadipocytes, adipocytes, macrophages, adventitial reticular cells, fibroblasts, and endothelial cells [1,2]. Preadipocytes and adipocytes constitute major components of the bone marrow stroma in vivo and are thought to play an active role in stromal cell-associated hematopoiesis [3–5]. However, the regulatory mechanisms involved in the interconversion between preadipocytes and adipocytes in vivo remain poorly understood.

We report here the isolation from the human bone marrow-derived stromal cell line KM-102 [6] of a cDNA encoding AGIF that inhibits the process of adipogenesis in cultured preadipocytes. The nucleotide sequence of the AGIF cDNA was revealed to be identical to that of a recently reported IL-11 cDNA [7], suggesting that AGIF/IL-11 may act as a multifunctional cytokine in the bone marrow microenvironment.

## 2. MATERIALS AND METHODS

### 2.1. *cDNA cloning*

A cDNA library was prepared in the Okayama-Berg expression vector (pcD) [8] from poly(A)$^+$RNA isolated from the KM-102 cells after stimulation for 3–14 h with phorbol myristate acetate (PMA, 10 ng/ml) and calcium ionophore A23187 (0.2 μM). The library was screened with $^{32}$P-labeled 15-mer oligonucleotide (5′-TAAATAAAT-AAATAA-3′) according to the method as described [9]. Among 6500 colonies screened, 33 positive clones were identified and then subjected to the second screening of functional expression cloning in COS-1 cells. A single clone, 20-2, that directed expression of suppressing activity for heparin-releasable lipoprotein lipase (LPL) in 3T3-L1 adipocytes [10] when introduced into COS-1 cells was isolated, and the plasmid was designated pcD-20-2. The cDNA insert prepared from pcD-20-2 was used as a hybridization probe for further screening of the same cDNA library consisting of 101,000 colonies, and three clones (20-I, J, and K) were obtained. The nucleotide sequences of these clones were determined by the dideoxy chain-termination method [11].

### 2.2. *Northern blot analysis*

Ten micrograms of poly(A)$^+$RNA was fractionated on a 1.0% agarose gel and blotted onto a nylon membrane [12]. The blot was hybridized with $^{32}$P-labeled cDNA prepared from pcD-20-2, washed as described [13], and exposed at −70°C to an X-ray film with an intensifying screen. The sizes of the AGIF/IL-11 mRNAs were estimated from the relative mobilities of RNA standards obtained from Bethesda Research Laboratories (Gaithersburg, MD, USA).

### 2.3. *Biological assay*

Differentiation leading to the adipocyte phenotype of 3T3-L1 cells was induced by a modification of the method as described [14]. 3T3-L1 cells were grown in Dulbecco's modified Eagle's medium containing 4.5 mg of glucose per ml, 10% fetal bovine serum (FBS), 10 mM HEPES (pH 7.2), 8 μg of d-biotin per ml, and 4 μg of pantothenic acid per ml (this medium is subsequently referred to as complete DMEM) in a 48-well tissue culture plate. After the cells had reached confluence, the medium was replaced with the adipocyte induction medium (complete DMEM containing 5 μg of insulin per ml, 1 μM dexamethasone, and 0.5 mM isobutyl-methylxanthine). Four days later the induction medium was replaced with the maintenance medium (complete DMEM containing 100 ng of insulin per ml). After two days of culture, the medium was replaced with fresh maintenance medium supplemented with several dilutions of conditioned medium of COS-1 cells transfected with pcD-20-2 by electroporation [15].

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After an 18-h culture period, activity of heparin-released LPL of the cells was determined by a modification of a previously reported method [14,16,17].

## 3. RESULTS AND DISCUSSION

### 3.1. *cDNA cloning and sequence analysis*

We isolated cDNA clones encoding human AGIF from a cDNA library prepared from poly(A)$^+$RNA isolated from PMA and A23187-treated KM-102 cells. The cDNA library was first screened with an oligonucleotide probe that can detect ATTTA repeats, because AUUUA motifs are found in the 3'-noncoding region of mRNAs for many inflammatory cytokines and some proto-oncogenes [18]. The selected cDNA clones were introduced into COS-1 cells, and the supernatants were tested for their ability to suppress LPL activity in cultured 3T3-L1 adipocytes. The 3T3-L1 cell line is a well-characterized experimental model for adipocyte differentiation and LPL activity is a marker of adipocyte differentiation. After the screening, one positive clone was obtained and designated 20-2. The conditioned medium of COS-1 cells transfected with pcD-20-2 not only suppressed the LPL activity of differentiated 3T3-L1 adipocytes but also inhibited the process of adipogenesis in 3T3-L1 preadipocytes as will be described elsewhere (manuscript in preparation). Therefore, we designated this cytokine as adipogenesis inhibitory factor (AGIF).

The cDNA insert of pcD-20-2 was used as a hybridization probe for further screening; and additional clones 20-I, J, and K were thus obtained. The nucleotide sequence of each of these four cDNA clones was fully or partially determined (Fig. 1). Clone 20-I was derived from a 2.6-kb transcript; and the other three clones came from a 1.3-kb transcript (Fig. 2). The size discrepancy of the two transcripts resulted from the alternative polyadenylation signals used. Therefore, both of the transcripts contain the same open reading frame of 597 nucleotides encoding a peptide of 199 amino acids. The AGIF cDNA and translated protein sequences were compared to other sequences in available sequence data banks. No significant similarity to any sequences deposited in the data banks was revealed, but we have found that the nucleotide sequence of the AGIF cDNA is identical to that of a recently reported human IL-11 cDNA [7].

### 3.2. *Northern blot analysis of AGIF/IL-11 mRNAs*

Northern blot analysis revealed that the two AGIF/IL-11 transcripts corresponding to 2.6 and 1.3 kb were expressed by PMA/A23187-treated KM-102 cells (Fig. 2). These two transcripts were constitutively expressed, but the level of each was increased after PMA/A23187 induction. No transcripts of similar size were detected in poly(A)$^+$RNA preparations from human liver, heart, and kidney. Alternative polyadenylation for the 2.6- and 1.3-kb transcripts was con-

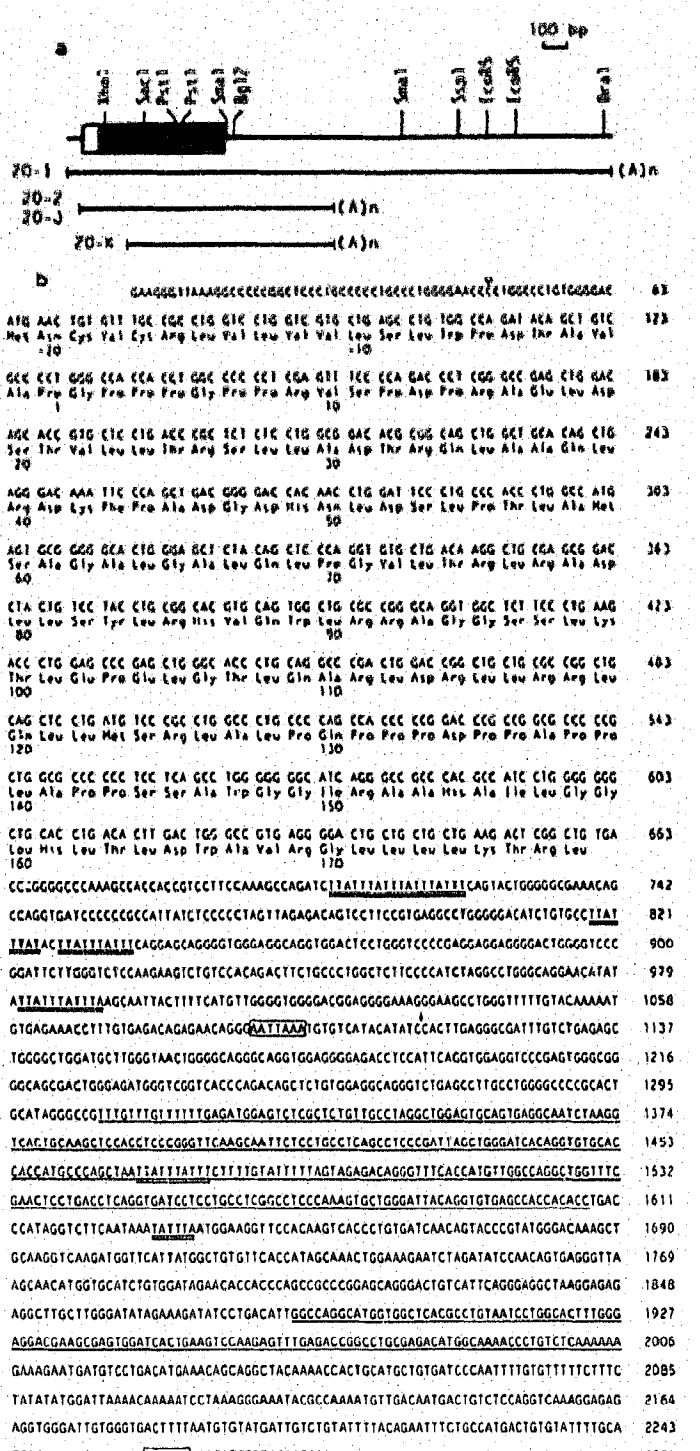


Fig. 1. (a) Schematic representation of human AGIF/IL-11 cDNA clones. The open box shows a signal peptide; and the solid box, the region coding for mature AGIF/IL-11. (b) Nucleotide and predicted protein sequences of AGIF/IL-11 cDNA clone 20-I. Amino acids are numbered starting at Pro (+1) of the mature AGIF/IL-11 protein sequence. The amino-terminal Pro was determined from recombinant AGIF purified from the culture supernatant of transfected COS-1 cells (manuscript in preparation). Inverted triangle, 5'-ends of clones 20-2 and 20-J; vertical arrow, poly(A) addition site of clone 20-2; boxes, presumptive polyadenylation recognition sites; underlines, Alu repetitive sequences; and double underlines, ATTTA mRNA destabilizer sequence elements [18].

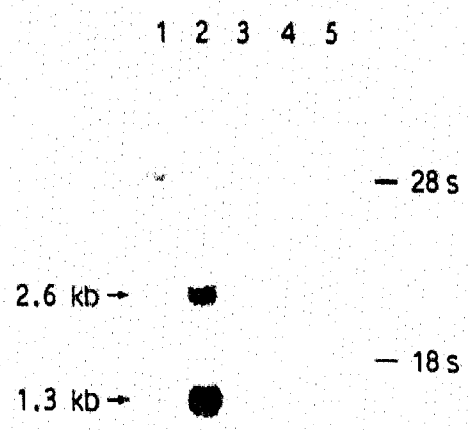


Fig. 2. Northern blot analysis of AGIF/IL-11 mRNA. Poly(A)$^+$RNAs isolated from uninduced (lane 1), PMA/A23187-induced (lane 2) KM-102 cells, and from human liver (lane 3), heart (lane 4), and kidney (lane 5) were subjected to blotting analysis.

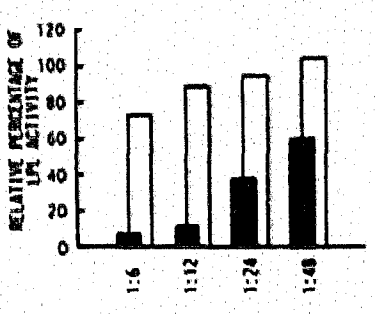


Fig. 3. Suppression of LPL activity of 3T3-L1 adipocytes by AGIF/IL-11. 3T3-L1 adipocytes were cultured in the maintenance medium in the presence of the indicated final dilutions of mock-transfected (open bars) or pcD-20-2-transfected (closed bars) COS-1-cell conditioned medium. After 18 h of culture, the heparin-released LPL activity was determined. LPL activity was expressed relative to the control (no addition of COS-1-cell conditioned medium), defined as 100%.

firmed by the result of Northern blot analysis by use of the clone 20-I-specific probe. Only the 2.6-kb band was detected in the KM-102-cell poly(A)$^+$RNA when the 200-bp SphI/DraI fragment, prepared from the 3′-noncoding region of the 20-I cDNA, was used as a probe (data not shown). So far, no functional specialization has been demonstrated for these mRNA classes produced by alternative polyadenylation, but one possibility remains, that they could be differentially utilized in different producer cells.

### 3.3. *LPL suppressing activity of AGIF/IL-11 in 3T3-L1 adipocytes*

The recombinant AGIF/IL-11 secreted from COS-1 cells transfected with pcD-20-2 suppressed the heparin-releasable LPL activity of differentiated 3T3-L1 adipocytes in a dose-dependent manner (Fig. 3). IL-11 was reported as a factor stimulating plasmacytoma proliferation and the development of immunoglobulin-producing B cells and megakaryocytes [7], but the findings presented here indicate that this factor may play a role in the regulation of adipocyte differentiation in the bone marrow hematopoietic microenvironment. The KM-102 cell itself is not thought to be a preadipocyte, because it does not differentiate into an adipocyte even in the induction medium [6]. Therefore, AGIF/IL-11 may function as an adipogenic antagonist in the bone marrow microenvironment in a paracrine manner. However, an autocrine role of AGIF/IL-11 cannot be excluded, because expression of AGIF/IL-11 in bone marrow preadipocytes and/or adipocytes has not yet been studied.

There are some reports on the relationship between the stage of differentiation of bone marrow-derived adipocytes and their hematopoiesis-supporting activity. This activity, through cell-to-cell interaction and/or secretion of colony-stimulating factors, has been reported to be higher in the preadipocyte state than in the adipocyte state in some cell lines [5,19]; although there is conflicting evidence that hematopoietic activity is not affected by terminal adipocyte differentiation in another cell line [20]. So far, other cytokines with the ability to inhibit adipogenesis have been reported: interferon [21,22], IL-1 [16,23], tumor necrosis factor [22,24,25], transforming growth factor beta (TGF-$\beta$) [26,27], leukemia inhibitory factor (LIF) [28], and IL-6 [29]. Among these, IL-1, IL-6, LIF, and TGF-$\beta$ are expressed in the bone marrow stromal cell lines. Therefore, these cytokines including AGIF/IL-11 may positively play a role in stromal cell-associated hematopoiesis through their regulatory action on adipocyte differentiation.

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