Characterization of Structural Domains of Human Osteoclastogenesis Inhibitory Factor*

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Osteoclastogenesis inhibitory factor (OCIF) is a heparin-binding secretory glycoprotein that belongs to the tumor necrosis factor receptor (TNFR) family. OCIF is present both as a 60-kDa monomer and a disulfide-linked homodimer. We attempted to characterize the seven structural domains of OCIF by determining the capabilities of various OCIF mutants to inhibit osteoclastogenesis, to interact with heparin, and to form dimers. We also examined a potential of domains 5 and 6, death domain homologous regions (DDHs), for inducing cell death by expressing OCIF/Fas fusion proteins. Our results show that: (i) the N-terminal portion of OCIF containing domains 1–4, which have structural similarity to the extracellular domains of the TNFR family proteins, is sufficient to inhibit osteoclastogenesis; (ii) a heparin-binding site is located in domain 7, and affinity for heparin does not correlate with the inhibitory activity; (iii) Cys-400 in domain 7 is the residue responsible for dimer formation; and (iv) the C-terminal portion containing domains 5 and 6, DDHs, has a high potential for mediating a cytotoxic signal when it is expressed in cells as an OCIF/Fas fusion protein in which the transmembrane region of Fas is inserted in front of DDHs.

In the vertebrate, homeostasis and remodeling of bone are by strictly controlled by mostly unrevealed mechanisms. Much effort has been made to clarify the mechanisms, and several protein factors were found to participate in bone homeostasis (1–3). Recently, we isolated one such factor termed osteoclastogenesis inhibitory factor (OCIF)1 from the conditioned medium of human embryonic lung fibroblasts, IMR-90 (4). Both a 60-kDa monomer and a disulfide-linked homodimer are present in the conditioned medium, and the two forms have similar specific activity in inhibition of osteoclast formation in vitro (4). However, the mechanism by which OCIF inhibits osteoclastogenesis is not yet known.

Based on the partial amino acid sequence, cDNA for human OCIF was molecularly cloned. The amino acid sequence deduced from the nucleotide sequence of OCIF cDNA predicted that it consists of 401 amino acid residues, including a putative 21-amino acid residue signal sequence (5). The nucleotide sequence analysis has revealed that OCIF is identical to osteoprotegerin (6). OCIF has seven major domains (domains 1–7) and has overall similarity to proteins of the tumor necrosis factor receptor (TNFR) family, although OCIF lacks an apparent transmembrane region (5, 6). Domains 1–4 are cysteine-rich structures with a characteristic of extracellular domains of the TNFR family proteins. Domains 5 and 6 share structural features with “death domains” of TNFR 1, Fas, DR 3 (also designated as Apo 3, Wal 1, and TRAMP), the TRAIL receptor, and the several recently identified cytoplasmic proteins mediating apoptosis (5, 7–17). However, unlike previously characterized death domains, two death domain homologous regions (DDHs), domains 5 and 6 of OCIF, exist in extracellular environments, because OCIF is secreted into conditioned medium. Domain 7, which does not resemble any protein motifs characterized thus far, consists of 50 amino acid residues and has a relatively high net positive charge; it contains eight basic amino acid residues (Lys and Arg) and only one acidic residue (Glu).

To determine which residue(s) or domain(s) is/are involved in the in vitro biological activity, binding to heparin, and dimer formation, we generated and characterized various mutants of OCIF. We also examined the potential of domains 5 and 6 for mediating cell death by overexpressing chimeric proteins in which portions containing the transmembrane domain derived from Fas were inserted into OCIF.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strain, Cell Lines, and Culture—**Escherichia coli DH5a (Life Technologies, Inc.) was used to propagate and amplify plasmids.

**Construction of Plasmids and Expression of Mutants of OCIF—**Mammalian expression plasmid pCEP4 (CLONTECH) was used for expression of OCIF mutants, Fas, and OCIF-Fas chimeric proteins. Full-length OCIF cDNA was subcloned into the XhoI and BamHI sites of pCEP4 to yield pCEP4-OCIF in which the cDNA is expressed under the control of the cytomegalovirus promoter. Human Fas cDNA (18) was amplified by PCR using Ex Tag polymerase (Takara Shuzo) and primers 5'-TCTTTCACTTCGGAGGATTG-3' (sense) and 5'-TCTAGAC-D1, kb, kilobase pair(s).

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purification of OCIF antibody was purified from serum of the rabbits using protein A-Sepharose chromatography. The affinity of wild-type OCIF was determined by measuring tartaric acid-resistant acid phosphatase (TRAP) activity in the medium, aliquots of conditioned medium were collected. The concentration of the mutant in each fraction was determined by ELISA employing rabbit anti-OCIF polyclonal antibody.

Western Blotting—Proteins were separated on SDS-polyacrylamide gel (10% or 13%) electrophoresis. Rainbow-colored molecular weight markers (Bio-Rad) were used as standards. Proteins were blotted onto Polyvinylidene difluoride (Bio-Rad) and examined under a phase-contrast microscope, and the percentage of round-shaped blue cells versus total blue cells was calculated. For the assay of lactate dehydrogenase activity in the medium, aliquots of conditioned medium were removed before fixing the cells. Lactate dehydrogenase activity was measured using a colorimetric kit (Shino-test, Tokyo, Japan). DNA fragmentation assay was performed as described previously (20) using cells cultivated for 20 h after transfection.

RESULTS

Construction of Vectors and Expression of OCIF Mutants—A schematic representation of OCIF and mutants of OCIF used in this work is shown in Fig. 1. We constructed a series of expression vectors and transduced them into 293-EBNA cells to produce deletion mutants, ΔD1, ΔD2, ΔD3, ΔD4, ΔD5, ΔD6, ΔD7, and ΔD8. We also constructed expression vector for C-terminal truncation mutants, ΔD67 and ΔD67. Furthermore, to identify a residue responsible for dimer formation of OCIF, a series of Cys to Ser mutants, C195S, C202S, C277S, C319S, and C400S, in which each Cys residue in domains 5, 6, and 7 was replaced with Ser, and a deletion mutant, ΔCL, which lacks two C-terminal amino acid residues, Cys-400 and Leu-401, were prepared. All but two mutants (ΔD1 and ΔD2) were detected in

ΔD2, and ΔD3 by XhoI/Ndel; ΔD4 by XhoI/SphI; ΔD5, C195S, C202S and C277S by Ndel/SphI; ΔD6 and C319S by Ndel/BstEI; ΔCL and C400S by SphI/BatEII; ΔD7 by SphI/BamHI; and ΔD67 and ΔD67 by NdeI/BamHI and subsequently with 8 ml of 0.15 M NaCl containing 0.1% CHAPS, proteins were eluted from the column with 0.2 M acetic acid. The fusion proteins were confirmed by SDS-PAGE and the fusion proteins were sequenced from the N-terminus end.

**Table 1**

| Mutant          | Expression vector | Protein expression |
|-----------------|-------------------|--------------------|
| ΔD1             | pCEP4-ΔD1         | 100%               |
| ΔD2             | pCEP4-ΔD2         | 100%               |
| ΔD3             | pCEP4-ΔD3         | 100%               |
| ΔD4             | pCEP4-ΔD4         | 100%               |
| ΔD5             | pCEP4-ΔD5         | 100%               |
| ΔD6             | pCEP4-ΔD6         | 100%               |
| ΔD7             | pCEP4-ΔD7         | 100%               |
| ΔD8             | pCEP4-ΔD8         | 100%               |

**Table 2**

| Mutant          | Expression vector | Protein expression |
|-----------------|-------------------|--------------------|
| ΔD1             | pCEP4-ΔD1         | 100%               |
| ΔD2             | pCEP4-ΔD2         | 100%               |
| ΔD3             | pCEP4-ΔD3         | 100%               |
| ΔD4             | pCEP4-ΔD4         | 100%               |
| ΔD5             | pCEP4-ΔD5         | 100%               |
| ΔD6             | pCEP4-ΔD6         | 100%               |
| ΔD7             | pCEP4-ΔD7         | 100%               |
| ΔD8             | pCEP4-ΔD8         | 100%               |

**Table 3**

| Mutant          | Expression vector | Protein expression |
|-----------------|-------------------|--------------------|
| ΔD1             | pCEP4-ΔD1         | 100%               |
| ΔD2             | pCEP4-ΔD2         | 100%               |
| ΔD3             | pCEP4-ΔD3         | 100%               |
| ΔD4             | pCEP4-ΔD4         | 100%               |
| ΔD5             | pCEP4-ΔD5         | 100%               |
| ΔD6             | pCEP4-ΔD6         | 100%               |
| ΔD7             | pCEP4-ΔD7         | 100%               |
| ΔD8             | pCEP4-ΔD8         | 100%               |

**Table 4**

| Mutant          | Expression vector | Protein expression |
|-----------------|-------------------|--------------------|
| ΔD1             | pCEP4-ΔD1         | 100%               |
| ΔD2             | pCEP4-ΔD2         | 100%               |
| ΔD3             | pCEP4-ΔD3         | 100%               |
| ΔD4             | pCEP4-ΔD4         | 100%               |
| ΔD5             | pCEP4-ΔD5         | 100%               |
| ΔD6             | pCEP4-ΔD6         | 100%               |
| ΔD7             | pCEP4-ΔD7         | 100%               |
| ΔD8             | pCEP4-ΔD8         | 100%               |

**Table 5**

| Mutant          | Expression vector | Protein expression |
|-----------------|-------------------|--------------------|
| ΔD1             | pCEP4-ΔD1         | 100%               |
| ΔD2             | pCEP4-ΔD2         | 100%               |
| ΔD3             | pCEP4-ΔD3         | 100%               |
| ΔD4             | pCEP4-ΔD4         | 100%               |
| ΔD5             | pCEP4-ΔD5         | 100%               |
| ΔD6             | pCEP4-ΔD6         | 100%               |
| ΔD7             | pCEP4-ΔD7         | 100%               |
| ΔD8             | pCEP4-ΔD8         | 100%               |

**Table 6**

| Mutant          | Expression vector | Protein expression |
|-----------------|-------------------|--------------------|
| ΔD1             | pCEP4-ΔD1         | 100%               |
| ΔD2             | pCEP4-ΔD2         | 100%               |
| ΔD3             | pCEP4-ΔD3         | 100%               |
| ΔD4             | pCEP4-ΔD4         | 100%               |
| ΔD5             | pCEP4-ΔD5         | 100%               |
| ΔD6             | pCEP4-ΔD6         | 100%               |
| ΔD7             | pCEP4-ΔD7         | 100%               |
| ΔD8             | pCEP4-ΔD8         | 100%               |
the conditioned medium by Western blot analysis (see below) employing anti-OCIF polyclonal antibody. OCIF mutants were quantified by ELISA using anti-OCIF polyclonal antibody. ΔD1 and ΔD2 were not secreted at levels detectable by Western blotting or ELISA. The concentration of most of the mutants in their conditioned media ranged from 200 ng/ml to 2 μg/ml. Accurate determination of inhibitory activity of ΔD6 was impossible due to poor productivity (~50 ng/ml).

The N-terminal Portion of OCIF Containing Domains 1–4 Is Sufficient to Inhibit Osteoclastogenesis—Fig. 2A shows the osteoclastogenesis inhibitory activity of the deletion mutants measured based on the inhibition of TRAP-positive osteoclast-like cell formation in the cocultures. Although wild-type OCIF inhibited the osteoclast-like cell formation in a dose range of 5 to 40 ng/ml (half-maximal inhibitory dose (ID₅₀) of ~6 ng/ml), ΔD3 and ΔD4 failed to inhibit the osteoclast-like cell formation at concentrations 35 and 40 ng/ml, respectively. In contrast, ΔD5 retained the inhibitory activity with an ID₅₀ of ~15 ng/ml. Furthermore, ΔD7 had a specific activity comparable to that of wild-type OCIF. A C-terminal truncation mutant ΔD67, which lacks domains 6 and 7, possessed the osteoclastogenesis inhibitory activity, although potency in the inhibitory activity was considerably lower than that of wild-type OCIF (an ID₅₀ of 10 ng/ml) (Fig. 2A). For the accurate determination of the inhibitory activity of ΔD567, which lacks DDHs entirely, we purified it using an anti-OCIF-antibody-immobilized affinity column. The purified ΔD567 inhibited osteoclastogenesis (Fig. 2B), indicating that truncation of domains 5–7 (consisting of the C-terminal 204 residues) does not abolish the biological activity. However, the potency of ΔD567 was approximately 10% of that of wild-type OCIF (Fig. 2B) as estimated from their ID₅₀. These results indicate that the N-terminal portion containing the first four domains is sufficient for exerting the osteoclastogenesis inhibitory activity in vitro.

Deletion of Domain 7 Decreases the Affinity of OCIF for Heparin—To examine the significance of the binding of OCIF to heparin in the inhibition of osteoclastogenesis, we analyzed the affinity of ΔD7, ΔD67, and ΔD567 for heparin by FPLC on HiTrap heparin column. Conditioned medium of the cells transiently expressing each OCIF mutant was loaded on the column and each mutant was eluted from the column with NaCl-containing buffer. Wild-type OCIF was eluted at NaCl concentrations of 0.55 M and 0.74 M, which correspond to those at which the monomer and the dimer form of OCIF are eluted, respectively (Fig. 3). ΔD7 was eluted as a single peak at an NaCl concentration of 0.24 M. Further truncation had only marginal effects on the binding of OCIF to heparin (Fig. 3). These three mutant proteins were eluted as single peaks, probably because they are present as monomers (see below). These results strongly suggest that domain 7, which occupies the C-terminal 50 amino acid residues, contains a heparin-binding site. The fact that deletion of domain 7 did not affect the inhibition of osteoclastogenesis (Fig. 2A) but significantly decreased the binding of OCIF to heparin (Fig. 3) indicates that binding ability of OCIF to heparin does not correlate with its osteoclastogenesis inhibitory activity in vitro.

Domain 7 Is Involved in the Dimerization of OCIF—OCIF from IMR-90-conditioned medium is present as two forms, a monomer with an approximate molecular mass of 60 kDa and a disulfide-linked dimer with an approximate mass of 120 kDa (4). To identify a domain(s) responsible for the dimer formation, the capability of the domain deletion mutants to form dimers was analyzed using immunoblotting as shown in Fig. 4A. A protein with a mass of 80–100 kDa was detected for ΔD3,
D4, D5, and D6 as a major band. In contrast, D7 is present almost exclusively as a 55-kDa protein. Thus, D7 is present mainly as a monomer, while D3, D4, D5, and D6 are present in two forms, a monomer and a dimer (or a multimer) in the conditioned medium, suggesting that domain 7 is involved in the dimer formation. D67 and D567, both lacking domain 7, migrated as monomers as expected (Fig. 4A). Difference in size between D3 and D4 (Fig. 4A) is probably due to a different degree of glycosylation. Indeed, there are three potential N-glycosylation sites (Asn-X-Ser/Thr) in domain 3, whereas there is no such site in domain 4 (5, 6).

Cys-400 Is Responsible for the Dimer Formation—Since there is only one Cys residue (Cys-400) in domain 7 (5, 6), participation of the residue in the intermolecular disulfide-linkage was suspected. To confirm this, two mutants, one with substitution of a Ser residue for Cys-400 (C400S) and the other with a deletion of the two C-terminal amino acid residues Cys-400 and Leu-401 (ΔCL) were produced. As a control, a series of Cys to Ser mutants, C195S, C202S, C277S, and C319S, in which each Cys residue in domains 5 and 6 was replaced with a Ser residue, were generated. The mutants were transiently expressed in 293-EBNA cells, and the structure of the mutants was analyzed by Western blotting as shown in Fig. 4B. The results indicate that both C400S and ΔCL exist almost exclusively as a monomer with a mass of ~60 kDa (Fig. 4B). No monomer-form OCIF with a mass of ~60 kDa was detected in the conditioned medium of C195S, C202S, C277S, or C319S (Fig. 4B). These four mutants migrated even slower than the dimer form OCIF with a mass of ~120 kDa. The slower migrating bands may represent higher order multimers derived from unusual disulfide bonding. These results demonstrate that Cys-400 is responsible for the dimer formation of OCIF. Thus, we conclude that the 120-kDa protein detected in the conditioned medium of OCIF-producing cells is a homodimer consisting of two 60-kDa monomers linked together by an intermolecular disulfide bond between two Cys-400 residues.
C400S and ΔCL, which are present almost exclusively as a monomer in the conditioned medium, were as potent as wild-type OCIF in the inhibition of the in vitro osteoclast formation (Fig. 5). These results provide further evidence that formation of the dimer is not essential for exerting the in vitro osteoclastogenesis inhibitory activity. C195S, C202S, C277S, and C319S, which are present mainly as multimers as shown in Fig. 4B, maintained the biological activity (data not shown).

Overexpression in 293-EBNA Cells of Chimeric Proteins Consisting of OCIF and the Fas Transmembrane Domain Causes Apoptosis—We next asked whether domains 5 and 6 (DDHs) have a potential for mediating cytotoxic signals. For this purpose, we transfected plasmids encoding various OCIF/Fas fusion proteins together with pCH110, an expression plasmid for β-galactosidase, in 293-EBNA cells. The structure of OCIF, Fas, and their fusion proteins used in this experiment is schematically illustrated in Fig. 6A. The presence of mRNA derived from each chimeric construct in the transfected cells was confirmed by reverse transcriptase-PCR. Primers were designed to amplify a 424-bp fragment corresponding to the 5′ portion of OCIF mRNA. As shown in Fig. 6B, reverse transcriptase-PCR using RNA from the cells transfected with pCEP4-OCIF-Fas, pCEP4-TM-OCIF, or pCEP4-TM-OCIFAD567 generated the 424-bp fragment, but not with pCEP4 or pCEP4-Fas. HRP-labeled anti-OCIF polyclonal antibody specifically bound to the cells transfected with pCEP4-OCIF-Fas, pCEP4-TM-OCIF, or pCEP4-TM-OCIFAD567, but not with the empty vector or pCEP4-Fas (Fig. 6C). These results indicate that each chimeric cDNA was efficiently expressed, and the fusion products were translocated to the surface of the transfected cells. These cells were then stained with X-gal to examine the size and the shape of the cells harboring each expression plasmid. Microscopic examination of the cells transfected with pCEP4-Fas, pCEP4-OCIF-Fas, or pCEP4-TM-OCIF revealed that 30–60% of the blue cells were round and shrunken, showing signs of cell death (Fig. 7A). In contrast, when transfected with the empty vector, pCEP4-OCIF or pCEP4-TM-OCIFAD567, more than 90% of the blue cells retained the flat and adherent appearance (Fig. 7A). Lactate dehydrogenase activity in the conditioned medium of the cells transfected with pCEP4-OCIF-Fas or pCEP4-TM-OCIF was significantly higher than that transfected with the empty vector or pCEP4-TM-OCIFAD567 (Fig. 7B), showing that overexpression of OCIF-Fas or TM-OCIF induced cell death. The transfection efficiency was almost the same (approximately 40%) in all transfection experiments. Cytotoxic signal induced by OCIF-Fas was apparently stronger than that induced by Fas for a currently unknown reason. To examine whether the cell death was caused by apoptosis, we next analyzed the integrity of DNA in the cells transfected with the empty vector, pCEP4-OCIF-Fas or pCEP4-TM-OCIF. Cells transfected with pCEP4-OCIF-Fas or pCEP4-TM-OCIF showed severe fragmentation of DNA, a clear symptom of apoptosis, as compared with controls (Fig. 7C). These results demonstrate that OCIF is capable of triggering apoptosis when the Fas transmembrane region is inserted between cysteine-rich regions and DDHs and
that domains 5–7 have a potential comparable to the death domain of Fas in the induction of apoptosis.

**DISCUSSION**

OCIF belongs to the TNFR family, containing four cysteine-rich domains and two DDHs followed by a domain with a high net positive charge (5, 6). In addition, OCIF has some characteristics that not all of the TNFR family proteins possess: (i) it is a secretory protein with no apparent transmembrane region; (ii) it is present in two forms, a monomer and a dimer; and (iii) it interacts with heparin. In the present study, we examined which structural domains are involved in the inhibition of osteoclastogenesis, the binding to heparin, and the formation of the dimer. We also examined whether the two DDHs have potential for mediating the cytotoxic signal when a chimeric protein in which the Fas transmembrane region is inserted in front of DDHs is produced in cells. The results are summarized in Fig. 8.

By analyzing the osteoclastogenesis inhibitory activity of deletion and C-terminal truncation mutants, we found that the N-terminal portion containing domains 1–4 is sufficient to inhibit osteoclastogenesis in vitro. Domains 1–4 correspond to the extracellular cysteine-rich regions of the TNFR family proteins. For the TNFR family proteins, these regions protrude outside the cells and are involved in the interaction with their ligands. Even for the soluble form receptors, the cysteine-rich regions still have the ability to associate with their ligands. A naturally occurring secreted form of Fas lacking the transmembrane domain is present in patients with systemic lupus erythematosus, suggesting that the molecule works as an antagonist against Fas (22). A soluble form of TNFR consisting of the cysteine-rich region, which is generated by a proteolytic cleavage, inhibits the activity of both TNF-α and TNF-β (23–26). Thus, most of the soluble variants of the TNFR family proteins act as antagonists against the membrane-bound signaling receptors. This fact raises the possibility that OCIF competes with a currently unidentified receptor capable of triggering osteoclast formation, by binding to a ligand with a structural similarity to TNF (4).

Recently, Simonet et al. (6) have reported the isolation of a cDNA coding for osteoprotegerin, a protein identical to OCIF. They showed that the first four domains alone can exert its inhibitory activity in vitro by analyzing the biological activity of C-terminal truncation mutants (6). However, the biological activity of the mutants was not accurately determined in their study. We first found that conditioned medium of cells producing ΔD567 was capable of inhibiting osteoclastogenesis in a dose-dependent manner (data not shown). Then, the mutant was purified to homogeneity to determine the potency. By
analyzing the in vitro biological activity of the mutant, we concluded that the N-terminal portion of OCIF is sufficient to inhibit osteoclastogenesis, although the potency is approximately one tenth of that of wild-type OCIF (Fig. 2B).

Analysis of heparin binding capability of the mutants revealed that domain 7 is involved in heparin binding. Binding to heparin or heparin-like molecules is known to be important for such growth factors as basic fibroblast growth factor to function in vitro and in vivo (21). The affinity of OCIF for heparin, however, did not correlate with the in vitro biological activity. For some proteins, changes in heparin-binding capability affect stability, rate of clearance, and target cell specificity in vivo. For example, a point mutation in superoxide dismutase that caused reduction of affinity for heparin results in a 10-fold increase in its plasma concentration without affecting the specific enzymatic activity in vitro (27). Therefore, it is worth examining the possibility that affinity of OCIF for heparin may be of some physiological importance in vivo. Most of heparin binding sites of known growth factors consist of a cluster of basic amino acid residues (28). Although no apparent cluster of positively charged amino acid residues is present in domain 7, application of Edmundson's wheel model to residues 361–378 shows an α-helix in which Lys-361, Lys-368, Lys-369, Arg-372, and His-375 exist on one side of the helix and hydrophobic residues such as Leu, Val, Ile, or Phe on the opposite side (data not shown). Such localized basic residues may contribute to the binding of OCIF to heparin. This was supported by the results that the mutants with alanine substitutions for Lys-368, Lys-369, and Arg-372 in OCIF had a marked decrease in affinity for heparin.

Domain 7 is also responsible for the dimerization of OCIF. This finding was derived from Western blot analysis of the mutants (Fig. 4A). Subsequent study identified Cys-400 as the residue essential for the dimer formation (Fig. 4B). Substitution or deletion of Cys-400 did not affect the activity of OCIF (Fig. 5), confirming the previous observation that both the monomer and the dimer form OCIF have similar specific activity in inhibition of in vitro osteoclastogenesis (4). The dimerization by disulfide bridges is extremely important for other TNF receptor homologs to exert inhibitory activity. Myxoma virus T2 protein, a TNFR homolog, is secreted as both a monomer and a dimer, and they bind to rabbit TNF-α with a similar affinity. Interestingly, the dimer is a more potent TNF inhibitor (29). The significance of dimer formation of OCIF remains open for the further investigation.

Domains 5 and 6 have a homology to death domains that are involved in transmitting “death” signals when expressed in the cytoplasm of 293-EBNA cells. It has been reported that ectopic expression of death domain-containing receptor proteins including TNFR 1, Fas, DR3 (also known as Apo-3, Wsl-1, or TRAMP) and the TRAIL receptor lead to cell death via apoptosis (11–14). Although overexpression of OCIF does not cause cell death, a receptor type OCIF (TM-OCIF), which contains the transmembrane region of Fas between domains 4 and 5 of OCIF, possessed an ability to induce apoptosis in 293-EBNA cells (Fig. 7, A–C). Truncation of the DDHs prevented the cell death, strongly suggesting that domains 5 and/or 6 have a high potential for mediating the apoptosis. Analysis of the OCIF gene failed to identify any sequences encoding a potential membrane-spanning domain, suggesting that OCIF exists only in soluble form. Therefore, it is unlikely that OCIF triggers apoptosis in a manner similar to TNFR 1 or Fas. Instead, OCIF may induce apoptosis in an unknown fashion under certain conditions.

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