Regulation of Macrophage Migration Inhibitory Factor and Thiol-specific Antioxidant Protein PAG by Direct Interaction*

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Macrophage migration inhibitory factor (MIF) is an important mediator that plays a central role in the control of the host immune and inflammatory response. To investigate the molecular mechanism of MIF action, we have used the yeast two-hybrid system and identified PAG, a thiol-specific antioxidant protein, as an interacting partner of MIF. Association of MIF with PAG was found in 293T cells transiently expressing MIF and PAG. The use of PAG mutants (C52S, C71S, and C173S) revealed that this association was significantly affected by C173S, but not C52S and C71S, indicating that a disulfide involving Cys173 of PAG is responsible for the formation of MIF-PAG complex. In addition, the interaction was highly dependent on the reducing conditions such as dithiothreitol or beta-mercaptoethanol but not in the presence of H2O2. Analysis of the activities of the interacting proteins showed that the d-dopachrome tautomerase activity of MIF was decreased in a dose-dependent manner by coexpression of wild-type PAG, C52S, and C71S, whereas C173S was almost ineffective, suggesting that the direct interaction may be involved in the control of d-dopachrome tautomerase activity of MIF. Moreover, MIF has been shown to bind to PAG and it also inhibits the antioxidant activity of PAG.

Macrophage migration inhibitory factor (MIF) is a cytokine that plays an important role in the regulation of host immune and inflammatory response (1–6). MIF is different from other cytokines in that it is found preformed in MIF-expressing cells (7, 8). In addition, MIF has been proposed to catalyze chemical reactions. Structural studies of MIF have led to the suggestion that MIF bears a close architectural similarity to microbial enzymes such as 5-carboxymethyl-2-hydroxymuconate, 4-oxoacrotonate tautomerase, and chorismate mutase, even though these proteins share little homology in the amino acid sequence (9–12). On the other hand, based on the amino acid sequence homology and structural similarity of MIF with d-dopachrome tautomerase, which converts d-dopachrome methyl ester to 5,6-dihydroxyindole-2-carboxymethylster, a d-dopachrome tautomerase activity also has been proposed for MIF (13, 14). However, the physiological significance is currently unclear, because natural substrates of MIF have not yet been found. MIF was demonstrated to catalyze the keto-enol isomerization of both p-hydroxyphenylpyruvate and phenylpyruvate, a hydroxyphenylpyruvate tautomerase activity (15). More recently, MIF has been reported to possess a thiol-protein oxidoreductase activity (16), which involves the reduction of insulin and 2-hydroxyethylsulfide.

Several lines of evidence suggest that the intracellular redox regulation might be involved in a variety of cellular functions, including cell proliferation, differentiation, tumor promotion, and apoptosis (17, 18). Antioxidants govern the intracellular redox status. PAG, a known thiol-specific antioxidant, is a member of the peroxiredoxin (Prx) protein family, which was previously referred to as the alkyl hydroperoxide reductase/thiol-specific antioxidant family and is constitutively expressed in most human tissues, but its expression is higher in organs having a higher level of proliferation (19, 20). Many Prx proteins are highly conserved in a wide variety of mammalian species such as human, mouse, and bovine, suggesting a biological importance of this type of enzyme (20–22). Most Prx family members contain two conserved cysteines that correspond to Cys47 and Cys170 of yeast thioredoxin peroxidase. In yeast, both Cys47 and Cys170 were shown to be necessary for the formation of intermolecular disulfide bonds, and Cys47, but not Cys170, was the primary site of oxidation by H2O2 (23).

Here we show that PAG binds specifically to MIF in vivo, and we found that this interaction is dependent on the redox status in that the interaction was significantly affected under reducing conditions. Binding of PAG to MIF can repress the d-dopachrome tautomerase activity of MIF. Moreover, this binding resulted in the suppression of the antioxidant activity of PAG.

EXPERIMENTAL PROCEDURES

Cell Lines and Reagents—293T cells, a derivative of human kidney embryonal fibroblast-containing SV40 T antigen, were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin-streptomycin and 1 mM glutamine in an atmosphere of 5% CO2 at 37 °C (24). The anti-FLAG (M2) antibody, dithiothreitol (DTT), aprotinin, and phenylmethylsulfonyl fluoride were purchased from Sigma Chemical Co. The mouse hybridoma cell line producing anti-GST antibody was generously provided by Dr. D. S. Im (Korea Research Institute of Science and Technology, Taejon). Rabbit anti-PAG antibody was raised against a recombinant PAG produced in E. coli (24). Polyvinylidene difluoride membrane was purchased from Millipore Corp.

Yeast Two-hybrid Assay—A genetic screen using the yeast interaction trap was performed as previously described (25). The full-length sequence
human MIF was cloned in-frame into anLexA coding sequence to generate a bait plasmid, pPEG202-MIF. A human HeLa cDNA library in the pG4-5 plasmid was screened for proteins that interact with MIF using EGY48 yeast strain (Maio trp1 ura3-52 leu2::pLeu2-Aeq96ΔG-I-\(\text{UAS} \text{leu}2\)). Yeast transformation was performed by the lithium acetate method (14). Colonies were selected, and the resulting plasmid was recovered and sequenced with dideoxy sequencing according to the manufacturer's instructions (Amerham Pharmacia Biotech). A fish plasmid, pG4-5 harboring PAG, was transformed back into yeast along with the pJG4-5 plasmid. Positive clones were selected, and the cDNA inserts were re-screened for proteins that interact with MIF.

Cotransfection and in Vivo Interaction Assay—293T cells grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM sodium pyrophosphate, 25 mM sodium 4-phenylbutyrate, 50 mM sodium \(\beta\)-glycerophosphate, and 10 \(\mu\)g/ml leupeptin, 10 \(\mu\)g/ml aprotinin, 10 \(\mu\)g/ml pepstatin, 10 \(\mu\)M sodium fluoride, 2 \(\mu\)g/ml antipain, 1 \(\mu\)g/ml aminopeptidase, and 1 \(\mu\)g/ml trypsin inhibitor were pooled and concentrated with a vivaspin-6 concentrator (Vivaspin, Iboly, France) and rotated for 24 hr at 4 °C. Beads were washed three times with ice-cold phosphate-buffered saline and solubilized with 100 \(\mu\)l of lysis buffer (20 \(\mu\)M Hepes (pH 7.9), 10 \(\mu\)M EDTA, 0.1 M KCl, and 0.3 M NaCl) containing 0.1% Nonidet P-40, 10 \(\mu\)M/ml aprotinin, 10 \(\mu\)M/ml leupeptin, 10 M sodium fluoride, 2 \(\mu\)g/ml \(\alpha\)-antitrypsin, 2 \(\mu\)M sodium pyrophosphate, 25 \(\mu\)M sodium \(\beta\)-glycerophosphate, and 10 \(\mu\)M sodium 4-phenylbutyrate. The soluble phase was pelleted by centrifugation at 13,000 rpm for 15 min at 4 °C. Approximately 80 \(\mu\)l of the cleared lysates was mixed with 15 \(\mu\)l of glutathione-Sepharose beads (Amerham Pharmacia Biotech) and rotated for 2 hr at 4 °C. Beads were washed three times with the lysis buffer. The bound proteins were eluted by boiling in SDS sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and then transferred to polyvinylidene difluoride membranes. The membranes were probed with an anti-FLAG (M2) antibody and then developed using an enhanced chemiluminescence (ECL) detection system (Amerham Pharmacia Biotech).

Expression Constructs—The eukaryotic glutathione S-transferase (GST) expression vector (pEBG) and pFLAG-CMV-2 vector with a FLAG epitope were obtained as described previously (25). For expressing proteins in mammalian cells, full-length MIF and PAG and their mutants were cloned into pEBG and pFLAG-CMV-2. The BamHI/NotI and HindIII/Xhol fragments of MIF cloned in pYESTrp (Invitrogen) were used to generate pEBG-WT-MIF and pEBG-WT-MIF, respectively. pEBG-WT-MIF-pAG harboring was constructed in several steps. We first cloned the pGEM-T vector (Promega), and each of the pBacPAK9-PAG mutants were digested with RI/\(I\) and subcloned into pEBG. The binding of MIF was subsequently analyzed using Western blot analysis shows that PAG was precipitated (Fig. 1A), indicating that MIF interacts with PAG specifically in the yeast two-hybrid system (data not shown). To determine whether MIF and PAG interact in vivo, we performed cotransfection experiments using GST- and FLAG-tagged eukaryotic expression vectors. In these experiments, the wild-type plasmid and MIF were coexpressed as a GST fusion protein and a FLAG-tagged protein in 293T cells, respectively. The interactions of FLAG-tagged MIF proteins to the GST-PAG fusion proteins were analyzed by immunoblotting with an anti-FLAG antibody. As shown in Fig. 1A, the MIF was detected in the coprecipitate only when coexpressed with the GST-PAG but not with the control GST alone. These observations support the results of the two-hybrid assay and demonstrate that MIF physically interacts with PAG specifically in the yeast two-hybrid system (data not shown).

In Vivo Association of MIF and PAG

**RESULTS**

**MIF and PAG Physically Interact with Each Other in Mammalian Cells**—In an effort to identify proteins that interacts with MIF, the yeast two-hybrid system was employed using the LexA DNA-binding domain MIF as a bait. From 3 \(\times\) 10\(^5\) individual transformants in a human HeLa cDNA library, ~100 clones specifically interacted with MIF. DNA sequencing and a BLAST algorithm of the positive clones revealed that 20 of 54 clones were found to encode partial or full-length sequences of PAG (accession number X67951). To verify the interaction specificity of PAG, the PAG library plasmid was rescued from the gal4-deprived dependent Leu ‘\(\text{lacZ}\)’ yeast and reintroduced into the original selection MIF bait strain as well as the other strains containing nonspecific baits available in our laboratory. From transformants, PAG cDNA was found to interact with both MIF and PAG but not with the other baits tested, indicating that MIF interacts with PAG specifically in the yeast two-hybrid system (data not shown).

To determine whether MIF and PAG interact in vivo, we performed cotransfection experiments using GST- and FLAG-tagged eukaryotic expression vectors. In these experiments, the wild-type plasmid and MIF were coexpressed as a GST fusion protein and a FLAG-tagged protein in 293T cells, respectively. The interactions of FLAG-tagged MIF proteins to the GST-PAG fusion proteins were analyzed by immunoblotting with an anti-FLAG antibody. As shown in Fig. 1A, the MIF was detected in the coprecipitate only when coexpressed with the GST-PAG but not with the control GST alone. These observations support the results of the two-hybrid assay and demonstrate that MIF physically interacts with PAG specifically in the yeast two-hybrid system (data not shown).
In Vivo Association of MIF and PAG

The MIF-PAG Complex Formation Is Affected by Cysteine Residues of PAG—Alignment of the peroxiredoxin (Prx) family members revealed two highly conserved cysteine residues, which correspond to Cys47 and Cys170 in yeast thiol-specific antioxidant (TSA), and, especially, the N-terminal cysteine (Cys47 in yeast TSA) was well conserved in all family members (20, 21–23). To investigate the effect of conserved cysteine residues of PAG on the MIF-PAG complex, 293T cells were transiently cotransfected with wild-type GST-PAG and mutants C52S (corresponding to Cys47 of yeast TSA), C71S, C83S, and C173S (corresponding to Cys170 of yeast TSA), together with FLAG-tagged MIF, and the complex formation between MIF and PAG was tested by coprecipitation with glutathione-Sepharose beads followed by immunoblotting with an anti-FLAG antibody. As shown in Fig. 2, expression of C173S mutant resulted in a dramatic decrease in the complex formation, whereas wild-type PAG, C52S, C71S, and C83S did not influence the significant change in the complex formation. These results clearly demonstrate that the conserved Cys173 of PAG plays a critical role in the association of MIF with PAG. Furthermore, these strongly indicate that the in vivo association of MIF and PAG is not affected by PAG activity, because no significant difference in the interaction was observed in the presence of C52S mutant that lacks Cys47, which corresponds to Cys47, essential for TSA activity, of yeast TSA (23). As a control, expression levels of total PAG and MIF proteins were determined by immunoblotting with antibodies to GST and FLAG using the same blot and cell lysates, respectively, and the amount of PAG and MIF in all lanes was similar (Fig. 2, middle and bottom). These results indicate that the observed difference in the complex formation was not due to differences in PAG and MIF expression levels.

MIF-PAG Interaction Is Dependent on the Redox Status—
PAG, a thiol-specific antioxidant, is known to exist as a homo- or heterodimer using disulfide linkages of cysteine residues (24). Additionally, the conserved Cys67-X-X-Cys60 motif of MIF was shown to form an intramolecular disulfide bond and the oxidoreductase and macrophage activating activities of MIF were found to be dependent on the presence of the cysteines in this motif (16, 30, 31). These observations led us to investigate the importance of the redox status in MIF-PAG complex formation. The cell lysates from 293T cells transiently cotransfected with GST-PAG and FLAG-tagged MIF were treated with DTT, β-mercaptoethanol, or H2O2, and the MIF-PAG complexes were then coprecipitated with glutathione-Sepharose beads and analyzed by immunoblotting with an anti-FLAG antibody. As shown in Fig. 3A, reductants such as DTT and β-mercaptoeth-
anol markedly decreased the amount of coprecipitated MIF, whereas H$_2$O$_2$, an oxidant, did not, suggesting that the redox-dependent interaction of MIF with PAG can occur in vivo. As a control, the expression levels of the transiently expressed proteins were analyzed in total cell lysates (for FLAG-tagged MIF, data not shown) and coprecipitates (Fig. 3 A, c and d), and similar expression levels were found for all lanes. Furthermore, a complex formation between GST alone used as a control and MIF was not detectable (Fig. 3 A, b). The redox-dependent interaction of MIF and PAG in vivo was further confirmed by transient expression of FLAG-tagged MIF in 293T cells (Fig. 3B). Endogenous PAG was immunoprecipitated using anti-PAG antibody, and coimmunoprecipitation of MIF was analyzed by immunoblotting with an anti-FLAG antibody. As expected, under the reducing conditions the amount of coimmunoprecipitated MIF was also decreased (Fig. 3B, a). As a control, the same blot was incubated with an anti-PAG antibody showing that similar amounts of endogenous PAG were immunoprecipitated (Fig. 3B, b). Taken together, these data strongly suggest that the in vivo association of MIF and PAG requires the participation of cysteine residues.

**PAG Is a Negative Regulator of MIF Activity**—To establish whether MIF-PAG complex formation is involved in the regulation of functional specificities of both proteins, we first analyzed the effect of PAG on MIF activity using a D-dopachrome tautomerase assay. Human PAG contains two cysteines, at amino acid positions 71 and 83, in addition to the conserved Cys$^{52}$ and Cys$^{173}$ (19). To investigate whether PAG indeed influences the MIF activity and, if so, whether the conserved Cys$^{52}$ and Cys$^{173}$ are necessary for the regulation of MIF activity, we used PAG mutants in which each of the cysteine residues was individually replaced by serine. The corresponding recombinant mutant (C52S, C71S, and C173S) and wild-type PAG proteins were expressed in E. coli and purified from the soluble extract of the bacterial cells. Although wild-type PAG, C52S, and C71S decreased the D-dopachrome tautomerase activity of MIF in a dose-dependent manner (Fig. 4A and data not shown), we did not detect a significant decrease in MIF activity in the presence of various concentrations of C173S (Fig. 4B). In addition, similar results were obtained when MIF activity was measured by a D-dopachrome tautomerase assay.

**FIG. 2.** Effect of wild-type and mutant PAG proteins on MIF-PAG interaction. 293T cells were transiently transfected with the appropriate expression plasmids, and GST fusion proteins were purified on glutathione-Sepharose beads (GST purification) and resolved by SDS-PAGE, and visualized by ECL. A complex formation between MIF and PAG was determined by Western analysis using anti-FLAG antibody (top panel). The same blot was reprobed with an anti-GST antibody to demonstrate the coprecipitation of an equivalent amounts of the GST fusion proteins (middle panel), and the expression level of FLAG-tagged proteins in total cell lysates was analyzed by Western analysis using anti-FLAG antibody (bottom panel).

**FIG. 3.** Redox status-dependent interaction of MIF and PAG. A, FLAG-tagged MIF expression plasmid (FLAG-MIF) was transiently cotransfected into 293T cells with pEBG (b, d), as a GST control, or pEBG-PAG expression plasmid (GST-PAG) (a, c). Purified GST fusion proteins immobilized on the beads were treated with the indicated concentrations of DTT, β-mercaptoethanol (β-ME), or H$_2$O$_2$, and the binding was determined as described in Fig. 1. To verify the amount of GST fusion and FLAG-tagged proteins in the coprecipitates, the purified GST-fusion proteins were analyzed by immunoblotting with anti-FLAG (a, b) and anti-GST antibodies (c, d). B, effects of reductants (DTT and β-ME) and an oxidant (H$_2$O$_2$) on the interaction of MIF with endogenous PAG. 293T cells were transiently transfected with FLAG-MIF, and MIF-PAG complexes were then immunoprecipitated with an anti-PAG antibody and analyzed by immunoblotting with an anti-FLAG antibody (a). The amount of immunoprecipitated PAG was analyzed by anti-PAG antibody immunoblot (b).
This observation is in agreement with the in vivo binding data obtained with PAG mutants (see Fig. 2), showing that MIF is present in the C52S and C71S but not in the C173S. Taken together, these results indicate that the direct binding of PAG with MIF is very important for regulation of the D-dopachrome tautomerase activity of MIF and suggest that MIF activity is negatively regulated by PAG.

**MIF Is Also Required for Negative Regulation of PAG Activity**—To determine whether the MIF, an interacting partner of PAG, also regulates PAG activity using the same approach, His-tagged proteins, including MIF, wild-type PAG, and PAG mutants (C52S, C71S, C173S) were expressed and purified from *E. coli* BL21 cells. Purified His-tagged proteins were assessed by reducing SDS-PAGE and were all detected at the corresponding molecular sizes (data not shown). PAG belongs to the Prx protein family of thiol-specific antioxidants, and the standard assay for thiol-specific antioxidants is the protection of glutamine synthetase from inactivation by a thiol/Fe3+/O2 mixed-function oxidase system (27). In this assay, the recombinant wild-type PAG and C71S mutant showed similar antioxidant activities, whereas mutants C52S and C173S had no effect (data not shown). We next asked whether MIF could influence the antioxidant activity of PAG. Increasing amounts of MIF were added to 30 mg of wild-type PAG, which resulted in a 100% protection of inactivation of glutamine synthetase. As shown in Fig. 5, this shifts the protection value from 100% (PAG alone) to 0% (5 mg of MIF), and the inhibitory effect of MIF on the antioxidant activity of PAG is concentration-dependent. These results clearly indicate that MIF and PAG do not only interact physically, but that binding of MIF also inhibits the antioxidant activity of PAG.

**DISCUSSION**

The present study demonstrates that MIF interacts with PAG in vivo, and that the MIF-PAG interaction is dependent on the redox status and the cysteine residues of PAG. In addition, we found that both D-dopachrome tautomerase activity of MIF...
and antioxidant activity of PAG were negatively regulated by direct association of MIF with PAG.

MIF has been proposed as a novel cytokine containing a combined function both as a conventional cytokine and as an enzyme (1, 13, 16). Recently, several studies have reported a variety of enzymatic functions for MIF, including β-dopachrome tautomerase (13, 14), phenylpyruvate tautomerase (15), and a thiol protein oxidoreductase (16). β-dopachrome tautomerase was discovered during the study of melanin biosynthesis (13). This enzyme was found to have an amino acid sequence that is highly homologous with that of MIF. It has previously been shown that the N-terminal proline of MIF is required for the β-dopachrome tautomerase and phenylpyruvate tautomerase activities (32–34). However, based on the observed sequence homology with known thiol-protein oxidoreductases (35), it is conceivable that MIF may exhibit a cysteine-dependent enzymatic oxidoreductase activity and that this activity is dependent on the redox-active conserved sequence motif (Cys-X-X-Cys). Analysis of the amino acid sequence of MIF revealed that there was a conserved sequence motif consisting of Cys57-Ala-Leu-Cys60 that was found to be present in the catalytic center of thiol-protein oxidoreductases such as thioredoxin, protein disulfide isomerase, and glutaredoxin. Consistent with this fact, recently a critical role of the conserved cysteine sequence motif (Cys-X-X-Cys) in the oxidoreductase and macrophage-activating activities of MIF was reported (16). The existence of an intramolecular disulfide bridge was also demonstrated from studies of the conserved cysteine sequence motif, even though previous studies had shown that neither recombinant E. coli-derived MIF nor native MIF from natural cell sources contained an intramolecular disulfide structure (7, 16, 36). In addition, MIF was crystallized as a trimer of three identical subunits (11, 28, 29). This raises the possibility that MIF may heterodimerize with other cellular proteins in addition to its ability to homodimerize through disulfide linkages between cysteine residues, and that redox regulation may be also involved in the control of this association. To address this question, we sought to identify cellular target proteins that directly associate with MIF. In this study, we report isolation of PAG as an MIF-interacting protein. We performed the coprecipitation experiments of transiently expressed MIF and wild-type PAG, as well as PAG mutants, and demonstrated that MIF associates with wild-type PAG and PAG mutants tested, except for C173S, in mammalian cells (Fig. 2), suggesting that the conserved Cys173 of PAG plays a pivotal role in the formation of intermolecular disulfide linkages between cysteine residues of PAG and MIF. However, we cannot rule out the other possibility that the other conserved Cys52 is also involved in the intermolecular disulfide linkages, because we are able to detect a significant decrease in the association of MIF with C52S, although to a somewhat lesser extent, by coprecipitation studies (Fig. 2). To gain more insight into the roles of cysteine residues in the MIF-PAG association, we examined the in vivo binding of MIF and PAG under various redox conditions. As shown in Fig. 3, upon reducing conditions the association was remarkably decreased in a dose-dependent manner, suggesting that the in vivo association of MIF and PAG may be mediated through disulfide linkages of cysteine residues. Recent studies have suggested that MIF and thiol antioxidants could be bridged through inter-chain disulfides (24, 37, 38). It is necessary to determine whether the conserved cysteine residues of PAG could influence hetero- and homodimerization and whether this complex formation contributes toward the regulation of functional activities of interacting partners.

To understand how the interaction contributes to the activity of the respective proteins, the examination of how MIF or PAG binding modulates the interacting protein function seems to be important. As shown in this report, it can be concluded that PAG is a negative regulator of MIF activity. Moreover, we confirmed this observation with the PAG mutants such as C52S, C71S, and C173S. From these mutation experiments, all mutants, except for C173S, resulted in a decrease of β-dopachrome tautomerase activity of MIF (Fig. 4 and data not shown). These findings can be explained either by a physical binding of PAG to MIF or by an enzymatic function of PAG. However, the fact that C52S mutant, but not C173S, indeed decreases the MIF activity, together with our observation that both Cys52 and Cys173 are equally critical for the antioxidant activity of PAG (data not shown), does not favor the second model describing the importance of the enzymatic function of PAG in the regulation of MIF activity. In addition, it has recently been shown that several MIF residues near the N-terminal proline are perturbed upon addition of S-hexylglutathione and affected by p-hydroxyphenylpyruvate, a substrate for the phenylpyruvate tautomerase activity of MIF (33, 38). Because our studies reported here demonstrated that PAG could inhibit the β-dopachrome tautomerase activity of MIF, it is tempting to speculate that this effect is mediated through the perturbation of the residues surrounding the N-terminal proline on the three-dimensional structure of MIF by direct binding of PAG. Based on our observed results, we imagine that rather than direct contact with the residues surrounding the N-terminal proline, the conformational effect that is caused by intermolecular disulfide linkages between cysteine residues of PAG and MIF likely plays a role that is important in the regulation of MIF β-dopachrome tautomerase activity, probably by structural changes within the region near the N-terminal proline.

A recent study (30) demonstrated that Aop1, a human thioredoxin peroxidase, and cyclophilin18 do not only interact physically, but binding of cyclophilin18 also stimulates the antioxidant activity of Aop1, suggesting an important role for the direct interaction in the regulation of the antioxidant activity of thioredoxin peroxidases. To test a possible influence of binding of MIF on the enzymatic activity of PAG, we used a similar approach. As shown in Fig. 5, a dramatic decrease was observed in the antioxidant activity of PAG when increasing amounts of MIF were added to the PAG. This also indicates that MIF negatively regulates the PAG activity. In this regard, the mechanism of interaction of MIF and PAG will be the interest of future study. Further understanding of the mechanism of this interaction will result from the identification of other interacting proteins associated with MIF or PAG and detailed analyses of the binding region in the MIF-PAG interaction.

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