Cloning and Characterization of a Novel Oxidoreductase KDRF from a Human Bone Marrow-derived Stromal Cell Line KM-102*

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A cDNA clone coding for a novel oxidoreductase was cloned from a human bone marrow-derived stromal cell line KM-102. We screened a cDNA library constructed from the mRNA of KM-102 cells stimulated with phorbol 12-myristate 13-acetate and calcium ionophore A23187 using a 32P-labeled 15-mer synthetic oligonucleotide (5'-TAAATAAAATAAATAA-3'). This probe was designed as a complementary sequence to the three reiterated AUUUA sequences, which are contained in the 3'-untranslated regions of cytokine and some proto-oncogene mRNAs and correlate with rapid mRNA turnover. Then, we obtained one cDNA clone, and further sequence analysis revealed that it coded for a new protein exhibiting 30 to ~40% homology with glutathione reductase. By fusion protein analysis, this protein showed reducing activities on 2,6-dichlorophenol-indophenol and 5,5'-dithio-bis(2-nitrobenzoic acid) but only a weak reducing activity on oxidized glutathione. Although it lacked a stretch of hydrophobic amino acids in its N terminus, it was secreted by monkey kidney-derived COS-1 cells when we introduced the expression plasmid into them and also secreted by a human lung carcinoma cell line A549. Northern blot analysis revealed that the mRNA turnover of this protein was regulated by inflammatory stimuli in KM-102 cells. These results show that this protein may have scavenging enzyme properties and has its mRNA expression regulated in a similar fashion to cytokine genes or proto-oncogenes. Thus, we named it KDRF (KM-102-derived reductase-like factor), and KDRF may play a role in scavenging reactive oxygen intermediates, which are possibly toxic to cells, in response to inflammatory stimuli.

Molecular oxygen, utilized in the form of oxidizing agents in the metabolic pathway within cells, is reduced to form water (H₂O) after passing through a reactive oxygen intermediate (ROI)¹. Through the activation of a variety of ROI-producing enzymes in response to exogenous stimuli, ROIs are produced in all cells. The representative ROIs are superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (·OH)(1–3). Because excessive accumulation of ROIs is toxic (1, 4), the intracellular level of ROIs is tightly regulated by several small antioxidant molecules (e.g. reduced glutathione), which contain sulfhydryl groups, and ROI-scavenging enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase). Both prokaryotic and eukaryotic cells have inducible defenses to counter oxidative damage (5–13). In fact, it has been reported that the mRNA of glutathione reductase (GR) or manganous superoxide dismutase was induced in human cells and cell lines by stimulation with lectin, tumor necrosis factor, or interleukin-1 (14, 15). But the mechanism(s) by which cells receive and respond to exogenous stimuli, including the induction mechanism(s) of the mRNAs of ROI-scavenging enzymes, has not yet been elucidated.

On the other hand, the control of transcription of many transiently expressed genes, such as cytokine genes and proto-oncogenes, has extensively been studied using exogenous stimuli. These experiments have shown that this class of mRNAs rapidly increased in cells by a signal(s) with the exogenous stimuli because it gives rise to a temporary block in specific mRNA degradation through protein synthesis (16). But the accumulation of the mRNAs is not maintained for a long time, and after receiving the signal, the amount of mRNAs in the cells rapidly decreases. A common feature of these rapid turnover mRNAs is that they all have reiterated or dispersed AUUUA sequences in their 3'-untranslated regions (17), and several studies have demonstrated that the presence of the reiterated or dispersed AUUUA sequences in the 3'-untranslated regions of the mRNAs correlated with rapid mRNA degradation (17–20). Thus, this class of mRNAs is posttranscriptionally regulated, and the AUUUA sequences are believed to be involved in the selective degradation of transiently expressed mRNAs.

In the case of scavenging enzymes, although mRNAs of GR and manganous superoxide dismutase also transiently increase with exogenous stimuli, their mRNAs do not have such reiterated or dispersed AUUUA sequences in their 3'-untranslated regions (21, 22), except that mRNA of human catalase has three dispersed AUUUA sequences in its 3'-untranslated region (23). Therefore, the relationship between such scavenging enzymes and the AUUUA sequences has yet to be discussed.

In this report, we describe the isolation and characterization of a cDNA clone encoding a novel oxidoreductase from a human bone marrow-derived stromal cell line KM-102 (24) using a complementary probe to the AUUUA sequences. We named it KDRF (KM-102-derived reductase-like factor) and demonstrated that this molecule may have scavenging enzyme properties and has its mRNA expression regulated in a similar fashion to cytokine genes or some proto-oncogenes also. The findings suggest that KDRF may play a role in scavenging ROIs, especially in response to inflammatory stimuli.
ExPERIMENTAL PROCEDURES

Preparation of Poly(A)$^\text{+}$ RNA from KM-102 Cells—Human stromal cell line KM-102 was a kind gift from Drs. K. Harigae and H. Handa. KM-102 cells were cultured with Iscove's modified minimum essential medium (Boehringer Mannheim) supplemented with 10% heat-inactivated fetal bovine serum in plastic culture dishes with a diameter of 15 cm. After growing the cells confluent, phorbol 12-myristate 13-acetate (PMA) (Sigma) and calcium ionophore A23187 (Sigma) were added to the culture at 10 ng/ml and 0.2 µM, respectively. After cultivation for 3, 6, or 14 h, total RNA for each was extracted by the guanidine thiocyanate extraction method (25). Six hundred µg of the total RNAs were used for Northern blot analysis.

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FIG. 2. The restriction maps, nucleotide sequence, and predicted amino acid sequence of the pUCKM31-7 cDNA. a, restriction endonuclease cleavage maps of the cDNAs of pUCKM31-7 and pcD-31. The darkened region represents the open reading frame. b, combined nucleotide sequence and deduced amino acid sequence of the insert cDNA of pUCKM31-7 and pcD-31. The numbers to the left and below each line refer to the nucleotide positions and amino acid positions, respectively. Comparison of the pUCKM31-7 cDNA sequence with that of the pcD-31 cDNA sequence revealed that the former sequence lacks a poly(A) tail. The probable methionine initiation codon is used for numbering the amino acids, and amino acids in the open reading frame following this methionine are indicated by double underlines. The vertical arrow marks the 5' end of clone pcD-31. The presumptive polyadenylation recognition site is marked with a single underline.
was selected, and this plasmid was named pSR31-7. Monkey kidney-derived COS-1 cells were transfected with pSR31-7. COS-1 cells were cultured in a flask (150 cm²) with Dulbecco’s modified Eagle medium (DMEM, Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal bovine serum. COS-1 cells were collected by trypsin-EDTA (Sigma) from the flask in which they were grown to semiconfluence and were washed twice with phosphate-buffered salts (PBS) (Sigma) at room temperature. COS-1 cells were passaged with the electroporation method (30). Each of the above-mentioned cell suspension and DNA solution were added into a Petri plate (9 cm), and this plasmid was named pSR31-7. Monkey kidney-derived COS-1 cells were transfected with pSR31-7 DNA in a reaction with 10 mg/ml of DEAE-Sepharose Fast Flow (Pharmacia Biotech Inc.) and eluted with 2.0 M NaCl. Western blot analysis was performed as described by Almouzni (31). Western blot analysis was conducted using ECL Western blotting detection reagent (Amersham Corp.). Rabbit antisera were raised against the purified pMAL31-7 DNA protein. These antisera were used for Western blot analysis. Molecular weight markers were purchased from Bio-Rad.

**Construction and Purification of Malaise Binding Protein (MBP) Fusion Protein—**pUCM31-7 DNA was digested with HindIII and SalI. After isolation and purification of the 3000-bp fragment containing the cDNA insert, the terminals were blunted using a DNA blunting kit. The fragment was then further digested with XhoI, and a fragment containing an open reading frame was purified. A fusion expression vector pMAL-c (New England BioLabs, Beverly, MA) was also digested with XhoI and SalI, followed by dephosphorylation with alkaline phosphatase. The dephosphorylated plasmid was then used for purification of recombinant protein. A fusion plasmid was reconstructed with HindIII (modified)—SalI fragment A). All of the PCR reactions were conducted using a Perkin-Elmer Cetus Thermal Cycler. The PCR conditions were: 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Following PCR, 8% polyacrylamide gel electrophoresis (PAGE) was performed to purify the amplified DNA product. After purification of this amplified fragment, we digested it with KpnI and SmalI. pML31-7 DNA were also digested with KpnI and SmalI, followed by dephosphorylation with alkaline phosphatase. The amplified fragment was then coupled with the dephosphorylated pML31-7 DNA in a reaction with T4 DNA ligase. *E. coli* DH10B (BRL) was transformed with this DNA, and the transformed strains were analyzed. We selected one strain and named this plasmid pMAL-K. It was confirmed that no abnormalities existed in the portion, in which the fragment was inserted, by analyzing the nucleotide sequence of this portion of pMAL-K. The fusion proteins produced in *E. coli* were purified with anion exchange affinity chromatography as recommended by the supplier (New England BioLabs). This cloning procedure is shown in Fig. 1.

**Cloning and Characterization of a cDNA for KDRF**

**Purification of Recombinant Protein and Amino Acid Sequence Analysis—**Serum-free conditioned medium (10 liters) of COS-1 cells transfected with the pSR31-7 expression plasmid DNA was dialyzed against 10 mM Tris-HCl, pH 9.0. The dialyzed sample was applied to a 10 ml of DEAE-Sepharose Fast Flow (Pharmacia Biotech Inc.) contained in an XK16/20 column (d 2.0 cm × 20 cm, Pharmacia Biotech Inc.) and eluted with a 0.0–0.5 M linear gradient of NaCl in the 10 mM Tris-HCl, pH 9.0, buffer. After collecting fractions (these fractions were monitored by Western blot) which were eluted at NaCl concentrations from 0.1 to 0.4 M, they were combined and dialyzed against 0.1 M Tris-HCl, pH 7.6, 5 mM EDTA, and 1 mM 2-mercaptoethanol (Sigma). Rabbit antisera were raised against the purified pML31-7 fusion protein. These antisera were used for Western blot analysis. Molecular weight markers were purchased from Bio-Rad.

**Cultivation of A549 Cells—**A human lung carcinoma cell line A549 was purchased from Dainihon Pharmaceutical Co. (Osaka, Japan), and A549 cells were cultured with DMEM supplemented with 10% fetal bovine serum. When the cells were grown to semiconfluence, the culture supernatant was removed, and the cells were washed with serum-free DMEM. Then, serum-free DMEM was added followed by culturing for an additional 3 days. The cell supernatant was then collected from the culture.

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pSRa31-7 coding protein, they were combined, and we electrophoresed it on SDS-PAGE under reducing conditions and transferred it electro- phoretically to a polyvinylidene difluoride film (ProBlot, Perkin-Elmer). The areas corresponding to the three bands of pSRa31-7 coding protein were cut out for protein microsequencing. Amino-terminal sequence determination was carried out on a gas-phase protein sequencer (PSSQ-10, Shimadzu Co.).

Oxidized Glutathione (GSSG), 2,6-Dichlorophenol-indophenol (DCIP), and 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB) Reduction Assay—Fusion protein derived from pMAL-K and protein from pMAL-c, which expresses only MBP, were purified with affinity chromatography and dialyzed against 0.01 M sodium phosphate, pH 7.5, and 5 mM EDTA (0.01 M phosphate buffer, subsequently referred to as PB). The concentrations of purified proteins were determined by Bio-Rad protein assay (Bio-Rad). All assays were done at room temperature using a Beckman DV 7500 spectrophotometer (Beckman Instruments, Fullerton, CA). Reducing activities were defined as Δ absorbance/min/mg of protein.

GSSG Reduction Assay—Ten μl of 10 mM GSSG (Boehringer Mannheim) in 0.01 M PB were added to 300 μl of 0.01 M PB containing the purified fusion protein, MBP, or yeast GR (yGR, Boehringer Mannheim). Then, 10 μl of 6.4 mM NADPH (Boehringer Mannheim) in 5 mM sodium bicarbonate were added to the mixture, and absorbance at 434 nm was monitored at room temperature for 3 min.

DCIP Reduction Assay—Three hundred μl of 50 μM DCIP (Sigma) in 0.01 M PB were added to 100 μl of 0.01 M PB containing the purified fusion protein, MBP, or yGR. Then, 10 μl of 6.4 mM NADPH in 5% sodium bicarbonate were added to the mixture, and absorbance at 550 nm was monitored at room temperature for 3 min.

DTNB Reduction Assay—Fifty μl of 10 mM DTNB (Wako Pure Chemical Industries, Osaka, Japan) in 0.01 M PB were added to 200 μl of 0.01 M PB containing the fusion protein, MBP, or yGR. Then, 10 μl of 6.4 mM NADPH in 5% sodium bicarbonate were added to the mixture, and absorbance at 412 nm was monitored at room temperature for 3 min.

RESULTS

Isolation of a cDNA Clone—An Okayama-Berg expression library constructed from poly(A)+ RNA of KM-102 cells, which had been stimulated with PMA (10 ng/ml) + A23187 (0.2 μM) for 3, 6, or 14 h, was screened with a synthetic oligonucleotide probe (5'-TAAATATAAATATAA-3'). Among the 6500 clones screened, 33 clones were hybridized with this probe. We selected one clone, pCD-31, and subjected it to further analysis. The pCD-31 clone was 491 bp in length excluding poly(A). However, Northern blot analysis of the poly(A)+ RNA from KM-102 cells revealed that the full-length mRNA of pCD-31 was approximately 3.9 kb. To obtain the full-length cDNA clone of pCD-31, we conducted a second screening. Among the 2 × 10^5 plaques screened, one positive clone was obtained (λ31-7). Following digestion of the λ31-7 DNA with EcoRI, the insert was recloned in the EcoRI site of pUC18, and the resultant plasmid was named pUCKM31-7. This cDNA clone was 3815 bp in length excluding a poly(A) tail and had a single open reading frame. The first ATG codon located at nucleotide 97 from the 5' end was followed by a 1647-nucleotide long open reading frame ending with an in-frame termination codon TGA at position 1744, and the open reading frame was, therefore, able to code for a 549-amino acid protein (Fig. 2b). The presumptive polyadenylation site, ATTAAA, was located 1851 nucleotides downstream from this TGA codon. A sequence, 5'-TTATTTTATTT-3', that is complementary to the synthetic probe was located 1580 nucleotides downstream from the termination codon (Fig. 2b). Such reiterated copies of AUUUA sequences are located in the 3'-untranslated regions of many cytokine and some proto-oncogene mRNAs, as mentioned previously.

Analysis of Deduced Amino Acid Sequence—Computer search on the SWISS-PROT and NBRF (PIR) protein sequence data banks showed the deduced amino acid sequence had 30 to ~40% identity with human glutathione reductase (hGR; EC 1.6.4.2)(21) and other species' GRs. A computer-aided comparison revealed 43.5% identity with probable GR of Caenorhabditis elegans (EC1.6.4.2)(32), 37.4% with Haemophilus influenzae (EC1.6.4.2)(34), 36.1% with Arabidopsis thaliana (EC1.6.4.2)(33), 36.3% with Pisum sativum (EC1.6.4.2)(35), 35.5% with human, 35.4% with Pseudomonas aeruginosa (EC1.6.4.2)(36), and 34.7% with Trypanothione reductase of Trypanosoma brucei (EC1.6.4.8)(37)(Fig. 3). An alignment of the amino acid sequence of hGR with pUCKM31-7 coding protein indicated an overall sequence identity of 35.5% (Fig. 4). The homology was absolute over a well conserved 10-amino acid sequence Leu^107-Gly-Gly-Thr-Cys-Val-Asn-Val-Gly-Cys^116, referred to as the active site, and a 9-amino acid sequence Ile^99-Gly-Gly-Ser-Gly-Gly-Leu-Ala^88, referred to as the FAD binding site. Rossmann fold structure (38, 39), which is the consensus sequence of the NADPH binding domains of GRs, was conserved in pUCKM31-7 coding protein Gly^249,-Ala-Ser-Tyr-Val-Ala-Leu-Glu-Cys-Ala-Gly-Phe-Leu-Ala-Gly-Ille-Gly^265. Arg^218 and Arg^224, which are important amino acid residues in determining the specificity for NADPH in hGR (39), were also conserved in the pUCKM31-7 coding protein (Arg^273 and Arg^278, respectively). Karplus et al. (40) identified six regions in the polypeptide chain of hGR which are involved in binding GSSG; these are residues 30–37, 59–64, 110–117, 339–347, 467–476, and 406. From an alignment of hGR with the pUCKM31-7 coding protein, the regions 30–37, 59–64, and 339–347 in hGR revealed high homology with the corresponding regions of the pUCKM31-7 coding protein, but the regions 110–117 and 467–476 in hGR revealed less homology, and the residue 406 was converted to Leu^461 in the pUCKM31-7 coding protein (Fig. 4). Arg^37, Arg^38, and Arg^47 in hGR are important residues for interaction with GSSG (39). Although Arg^47 in hGR was conserved as Arg^403 in the pUCKM31-7 coding protein, Arg^37 and Arg^38 in hGR were converted to Lys^471 and Glu^482 in the pUCKM31-7 coding protein, respectively. Human GR consists of two identical polypeptide chains, and Cys^90 in hGR is the residue forming a disulfide bridge with Cys^299 in the other peptide chain (41). But the residue corresponding to Cys^90 in hGR was converted to Ghu^144 in the pUCKM31-7 coding protein (Fig. 4). Although the pUCKM31-7 coding protein had a long N-terminal amino acid sequence in contrast to hGR, it lacked a stretch of hydrophobic amino acids in this region as well as hGR.

Expression in Mammalian Cells—To express the pUCKM31-7
Cys90 in hGR is indicated by an
for interaction with GSSG in hGR are indicated by
involved in binding with GSSG are
affected with pSR
determine their N-terminal amino acid sequences, we collected
of Reducing Activity—

pSR
determine its N-terminal amino acid sequence.

product with the highest molecular weight, we were unable to
protein probably corresponded to the second and third highest
weights, the 526 and 501 amino acid forms of pSR
acids, respectively. By Western blot analysis, three forms of
pSR
coding protein (KM-102-derived reductase-like factor).

Cloning and Characterization of a cDNA for KDRF

Expression and Transcriptional Regulation—Expression of KDRF. mRNA in various human cell lines and tissues
was studied by Northern blot analysis. As shown in Fig. 6a, KDRF mRNA was present in all of the cell lines that we
examined. Whereas it was found at low levels in HL-60, HeLa, K-562, MOLT-4, Raji, SW480, and G361 cells, it was present
at a high level in A549 cells. By Western blot analysis, we detected a single secreted form of KDRF in the conditioned me-

## Table I

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( \Delta ) absorbance/min/mg of protein*</th>
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<tbody>
<tr>
<td>pMAL-K</td>
<td>267.6</td>
</tr>
<tr>
<td>GSSG</td>
<td>0.3</td>
</tr>
<tr>
<td>DTNB</td>
<td>0.9</td>
</tr>
<tr>
<td>DCIP</td>
<td>5.3</td>
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* Assay mixtures lacking enzymes served as controls. Reducing activities of pMAL-c that express only MBP were the same as the controls.

![Fig. 5. Western blot analysis of the pUCKM31-7 coding protein (KDRF) secreted by transfected COS-1 cells and A549 cells.](http://www.jbc.org/)

Reducing activities of pMAL-K on oxidized glutathione (GSSG), 2,6-dichlorophenol-indophenol (DCIP), or 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB)

![Fig. 6. Northern blot analysis of KDRF mRNA expression.](http://www.jbc.org/)
Fig. 6. Northern blot analysis of KDRF mRNA. a, KDRF mRNA expression in various human tissues and cell lines. PBL represents peripheral blood lymphocytes. b, KDRF mRNA expression during stimulation of KM-102 cells. Poly(A)^+ RNAs were prepared from the KM-102 cells without stimulation (-) and after stimulation with PMA (10 ng/ml) + A23187 (0.2 μM), IL-1β (25 units/ml), or LPS (1 μg/ml) for 0.5, 1, 4, or 17 h. The ratio indicates the KDRF mRNA expression level in comparison with the level without stimulation. The KDRF mRNA was detected as a single band with 3.9 kb in length. Hybridization with a human β-actin probe is shown as a control for the integrity of the samples.

**DISCUSSION**

In this report, we described the cloning and characterization of a novel oxidoreductase KDRF from a human bone marrow-derived stromal cell line KM-102. The cDNA encoding this human protein was cloned using a unique synthetic oligonucleotide probe 5′-ATTTATTTATTT-3′, which is complementary to the three reiterated copies of AUUUA sequences.

From computer search analysis, KDRF exhibited 30 to 40% identity with hGR and other species' GRs. An alignment of its amino acid sequence with hGR showed an overall sequence identity of 35.5% (Fig. 3). The active site, the FAD binding site, and the Rossman fold structure (38, 39) were all conserved in KDRF (Fig. 4). Although two other important amino acid residues, Arg<sup>218</sup> and Arg<sup>224</sup>, were conserved in KDRF as Arg<sup>273</sup> and Arg<sup>278</sup>, respectively (Fig. 4), only three of the six regions that are involved in binding GSSG in hGR revealed high homology with KDRF. In particular, Arg<sup>277</sup> and Arg<sup>281</sup> in hGR were converted to Lys<sup>81</sup> and Glu<sup>82</sup> in KDRF, respectively (Fig. 4). By fusion protein analysis, KDRF revealed reducing activities on GSSG, DCIP, and DTNB but had a weak reducing activity on GSSG in contrast to yGR (Table I). The results suggest that many conversions of amino acids in KDRF, which are important for binding or interacting with GSSG, are the possible reason why KDRF showed only a weak reducing activity on GSSG in contrast to yGR. As shown in Table I, substrate specificities of KDRF were different from those of yGR. These results suggest that KDRF may have a natural substrate(s) other than GSSG. However, we cannot exclude the possibility that the MBP domain in the KDRF fusion protein might affect the activity. Therefore, we are now preparing for the measurement of the reducing activities on such substrates with recombinant KDRF protein, not a fusion protein, produced in E. coli.

From Northern blot analysis, mRNA coding for KDRF was detected in all of the tissues that we tested, although the expression levels varied in each tissue (Fig. 6a). We also examined the KDRF mRNA expression level in various human cell lines (Fig. 6c). The results showed low levels of expression in HL-60, HeLa, K-562, MOLT-4, Raji, SW480, and G361 cells but a high level in A549 cells. As described earlier, mRNA coding for KDRF was found to be at a low level in the lung. A549, which is a human lung carcinoma cell line, might have acquired the high expression mechanism(s) of KDRF in the process of carcinogenesis.

In the experiment in which the KDRF high expression plasmid was introduced into COS-1 cells, KDRF was secreted by the cells as proteins with three different N-terminal amino acid lengths, despite the fact that KDRF lacked a stretch of hydrophobic amino acids, called a signal peptide, in its N terminus (Fig. 5). The active site, the FAD binding site, and the Rossman fold structure (38, 39) were all conserved in KDRF (Fig. 4). Although two other important amino acid residues, Arg<sup>218</sup> and Arg<sup>224</sup>, were conserved in KDRF as Arg<sup>273</sup> and Arg<sup>278</sup>, respectively (Fig. 4), only three of the six regions that are involved in binding GSSG in hGR revealed high homology with KDRF. In particular, Arg<sup>277</sup> and Arg<sup>281</sup> in hGR were converted to Lys<sup>81</sup> and Glu<sup>82</sup> in KDRF, respectively (Fig. 4). By fusion protein analysis, KDRF revealed reducing activities on GSSG, DCIP, and DTNB but had a weak reducing activity on GSSG in contrast to yGR (Table I). We, therefore, suggest that many conversions of amino acids in KDRF, which are important for binding or interacting with GSSG, are the possible reason why KDRF showed only a weak reducing activity on GSSG in contrast to yGR. As shown in Table I, substrate specificities of KDRF were different from those of yGR. These results suggest that KDRF may have a natural substrate(s) other than GSSG. However, we cannot exclude the possibility that the MBP domain in the KDRF fusion protein might affect the activity. Therefore, we are now preparing for the measurement of the reducing activities on such substrates with recombinant KDRF protein, not a fusion protein, produced in E. coli.

From Northern blot analysis, mRNA coding for KDRF was detected in all of the tissues that we tested, although the expression levels varied in each tissue (Fig. 6a). We also examined the KDRF mRNA expression level in various human cell lines (Fig. 6c). The results showed low levels of expression in HL-60, HeLa, K-562, MOLT-4, Raji, SW480, and G361 cells but a high level in A549 cells. As described earlier, mRNA coding for KDRF was found to be at a low level in the lung. A549, which is a human lung carcinoma cell line, might have acquired the high expression mechanism(s) of KDRF in the process of carcinogenesis.

In the experiment in which the KDRF high expression plasmid was introduced into COS-1 cells, KDRF was secreted by the cells as proteins with three different N-terminal amino acid lengths, despite the fact that KDRF lacked a stretch of hydrophobic amino acids, called a signal peptide, in its N terminus (Fig. 5). We also detected a single secreted form of KDRF in the conditioned medium of A549 by Western blot analysis (Fig. 5). From these results, we concluded that KDRF might function as a reducing enzyme both inside and outside of the cell. Proteins with a defined extracellular function but lacking a signal sequence have been identified, which include IL-1β (42), basic fibroblast growth factor (43), adult T cell leukemia-derived factor (44), and ciliary neurotrophic factor (45). Although little is known about the mechanism(s) that allows their selective release or the KDRF secretion pathway, KDRF might also be a member of this class.
We confirmed that mRNA of KDRF had six AUUUA sequences, including two reiterated copies of AUUUA sequences, in its 3′-untranslated region (Fig. 2b). Northern blot analysis showed that its mRNA expression level in KM-102 cells increased 2 to 3 times after 4-h activation by various agents (PMA + A23187, human IL-1β, or LPS) and decreased thereafter, except in the case of PMA + A23187 (Fig. 6b). The turnover rate of KDRF mRNA in KM-102 cells was slow, but it has been reported that the reiterated AUUUA sequences might exert their effects through interaction with cell-specific factors and that some cell-specific differences exist in this mechanism (46). Thus, it is possible that mRNA of KDRF might more rapidly turn over in other cells than in KM-102 cells. However, it will be necessary for us to examine whether the expression of KDRF mRNA is regulated through the AUUUA sequences.

Considered together, KDRF is a new type of human protein that may have scavenging enzyme properties and has its mRNA expression regulated in a similar fashion to cytokine genes or proto-oncogenes. However, it is important to identify its inherent substrate(s), including not only small compounds but also peptides and proteins. It is necessary to elucidate the regulatory mechanism of its mRNA expression and its physiological role(s) as well. More detailed investigations should provide a deeper insight into these issues.

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Cloning and Characterization of a Novel Oxidoreductase KDRF from a Human Bone Marrow-derived Stromal Cell Line KM-102
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