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Cloning of hHRI, Human Heme-regulated Eukaryotic Initiation Factor 2α Kinase: Down-regulated in Epithelial Ovarian Cancers

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Protein synthesis is regulated in response to environmental stimuli by covalent modification, phosphorylating the components of the translational machinery. Phosphorylation of the α subunit of eIF-2 is one of the best-characterized mechanisms for down-regulating protein synthesis in higher eukaryotes in response to various stress conditions. One of mammalian eIF-2α kinases is a heme-regulated inhibitor kinase (HRI), which is activated by heme deficiency and plays an important role in translational control. In this work, we have analyzed the differentially expressed genes between epithelial ovarian cancer and normal ovary. We have screened a total of 1,408 genes isolated from a human dermal papilla cell cDNA library by cDNA array hybridization. Among many differentially expressed genes, eIF2α kinase, a heme regulated inhibitor was down-regulated in ovarian epithelium cancer. The down-regulation of hHRI was also confirmed in other ovarian cancer tissues by Northern blot hybridization. The hHRI gene is 2,887 bp in length and the amino acid sequence deduced from the cDNA clone encodes a protein of 630 amino acids with molecular mass of 73 kDa. It contains all 12 catalytic domains of the protein kinases with consensus sequences of the protein-serine/threonine kinases. The expression pattern of hHRI mRNA showed approximately 3.0 kb bands which were expressed ubiquitously in all human tissues examined, which indicates that eIF-2α kinase could play an important role in the translational regulation of nonerythroid tissues.

Keywords: cDNA Array; Human HRI Cloning; Ovarian Cancer.

Introduction

The phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF2α) is one of the best characterized mechanisms for down-regulating protein synthesis in mammalian cells in response to various stress conditions (Hershey, 1991; Samuel, 1993; Wek, 1994). The phosphorylation of eIF2α was first detected in rabbit reticulocyte lysates deprived of hemin. The absence of hemin resulted in the activation of a highly specific eIF2α kinase, called the heme-regulated inhibitor (HRI) (Chen and London, 1995). Phosphorylation of eIF2α in reticulocyte lysates results in the binding and sequestration of reversing factor (RF), also designated as the guanine nucleotide exchange factor or eIF2B, to make an RF · eIF-2α (P) complex. Since RF is required for the exchange of GTP for GDP in the recycling of eIF-2 and in the formation of the eIF-2 · Met-tRNAMet · GTP ternary complex, its unavailability results in cessation of the initiation of protein synthesis (Amesz *et al*., 1979; Matts and London, 1984).

Another mammalian eIF2α kinase, the double-stranded RNA-activated kinase PKR, is constitutively expressed in reticulocytes and inducible by interferon in other mammalian cells (Porud, 1995). At present, only these two distinct mammalian eIFα kinases have been cloned (Chen and London, 1995; Proud, 1995). A third eIF2α kinase, termed GCN2, has been characterized from *Saccharomyces cerevisiae* (Hinnebusch, 1997) and *Drosophila melanogaster* (Santoyo *et al*., 1997). It is activated by uncharged tRNA in response to amino acid starvation in yeast (Hinnebusch, 1997).

All three kinases phosphorylate eIF-2α on serine residue 51. The amino acid sequence surrounding serine 51 is highly conserved among the eIF-2α factors from human, rat, and yeast, and residues between 41 and 59 are identical (Cigan *et al*., 1989; Ernst *et al*., 1987). Although PKR, HRI, and GCN2 display a similar specificity with yeast and mammalian eIF-2α (Dever *et al*., 1992), some differences in specificity with peptide

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Abbreviations: GCN2, yeast general amino acid control eIF-2α protein kinase; hHRI, human heme-regulated inhibitor; PKR, double-stranded RNA-dependent eIF-2α protein kinase.

substrates are observed between the PKR and HRI enzymes (Proud *et al.*, 1991).

Activation of HRI in reticulocytes is mediated by various stimuli in addition to hemin deficiency, including heat shock, sulfhydryl reagents such as N-ethylmaleimide, oxidized glutathione, and heavy metal ions (de Haro *et al.*, 1983; Matts *et al.*, 1992; Palomo *et al.*, 1985). Native HRI appears to be a dimer composed of two 90 kDa polypeptides that may in part be disulfide-linked (Yang *et al.*, 1992). Binding of hemin to HRI promotes an intersubunit disulfide bond formation that may be involved in the negative regulation of HRI (Chen *et al.*, 1992). Heat shock proteins hsp90 and hsp70 also interact with HRI (Matts *et al.*, 1992). This interaction may be of regulatory significance. For example, hemin may regulate eIF-α HRI kinase activity by promoting the formation of an inactive HRI · hsp90 (p87) dimer (Mendez *et al.*, 1992). The regulation of HRI by its association to heat shock proteins appears to be similar to regulation of the steroid hormone receptor (Pratt, 1993).

In this study, we have screened the genes differentially expressed in ovarian cancer, using the strategy of cDNA array. One of the down-regulated genes in ovarian cancer was homologous to the mouse HRI (heme regulated inhibitor). We designated human HRI as hHRI and full-length cloned it for further study.

Materials and Methods

Construction of a cDNA library and sequence analysis A cDNA library was constructed by using a ZAP cDNA synthesis kit (Stratagene, La Jolla, USA) with 4 μg of poly A^+ RNA obtained from primary cultured human dermal papilla cells. The phage library was converted into a pBluescript phagemid cDNA library by *in vivo* excision using the ExAssist/SOLR system (Stratagene). Randomly selected clones were sequenced from the 5′ end of an insert using a T7 sequenase version 2.0 DNA sequencing kit (Amersham Pharmacia biotech.). Approximately 150 bases for each clone were compared with nonredundant GenBank data using BLASTN. Sequences were also translated and used to search the protein data base using the BLASTX sequence analysis program (Altschul *et al.*, 1990).

cDNA array preparation A total of 1,408 cDNAs were arrayed on the positive charged nylon membranes (Amersham Pharmacia biotech) in a 96-well format. Two identical blots were produced for each set of cDNA samples. The membrane was placed into the manifold (Bio-Rad, California, USA) and two hundred nanograms each of a denatured plasmid DNA sample per dot was loaded into the manifold. The membranes were dried in air, and fixed by UV crosslinking at 125 mJ using a UV Stratalinker 1800 (Stratagene).

Preparation of probe and hybridization Total RNAs were extracted from Ovarian tumor tissues and corresponding normal tissues from 13 patients by a modified acid guanidium thiocyanate/phenol/chloroform extraction method using the TRI reagent (Molecular Research Center Inc., Cincinnati, USA). Ten-micrograms of total RNA, which had been treated with human placental RNase inhibitor (Gibco BRL), were reverse-transcribed with an oligo-dT primer by using the SuperScript preamplification system (Gibco BRL). The first-stranded cDNAs were used for random primed radio-labelling (Megaprime DNA labelling system, Amersham Pharmacia biotech) to make the probe.

The DNA dot blots were hybridized with the radioactive probe using a ExpressHyb hybridization solution (Clontech, Palo Alto, USA), and washed according to the manufacturer's instructions. The membranes were analyzed after autoradiography.

Northern blotting analysis Ten micrograms of total RNA prepared from normal or cancer tissue were subjected to electrophoresis on a denaturing formaldehyde-agarose gel. The RNA was capillary transferred to a Hybond-N^+ membrane, baked for 2 h at 80°C under vacuum, and fixed by UV crosslink at 120 mJ using UV Stratalinker 1800 (Stratagene).

The purified 2.2 kb of a *Eco*RI-*Xho*I fragment of the h-HRI cDNA was used as a probe. The membranes were hybridized for 1 h at 68°C in ExpressHyb Solution (Clontech Laboratories Inc., USA) containing 2×10^6 cpm of radiolabelled probe per 1 ml of solution. After hybridization, the membranes were washed in 2× SSC/0.05% SDS at room temperature for 40 min and 0.1× SSC/0.1% SDS at 50°C for 40 min. The wet membrane was wrapped and exposed to X-ray film at −70°C for 4 d. Equal loading of mRNA was verified by rehybridizing the blots with a ^{32}P-labeled beta-actin cDNA probe.

Screening of a human dermal papilla cDNA library To obtain the full-length sequence of hHRI, the recombinant plaques of the dermal papilla and human prostate cDNA libraries (Clontech, Palo Alto, USA) were screened as described by Sambrook *et al.* (1989). Plaques were lifted onto nitrocellulose filters, and hybridized with 1×10^6 cpm/ml of a ^{32}P-labelled probe prepared by the Megaprime DNA labelling system (Amersharm, Buckinghamshire, England), in 5× Denhardt's solution, 0.1% SDS, and 100 μg/ml of denatured sonicated salmon sperm DNA, at 42°C overnight. The hybridized filters were washed at room temperature in 2× SSC (1× SSC; 150 mM sodium chloride, 15 mM sodium citrate, pH 7.0), 0.5% SDS for 20 min, and twice at 65°C in 1× SSC, 0.1% SDS for 1 h. Positive plaques were further purified by replating and hybridization. The isolated λ ZAP phagemid clones were converted into plasmids by *in vivo* excision using the ExAssist/SOLR system (Stratagene), the resulting plasmids pBluescript SK (−) having the cDNA of interest between the *Eco*RI and *Xho*I sites. Sequence analysis was performed as described previously.

Multiple Tissue Northern blots To examine the tissue-specific expression of hHRI, the expression of HRI mRNA in different human tissues was analyzed by Northern blot analysis using Multiple Tissue Northern (MTN) blots (Clontech). The MTN blots contained approximately 2 μg of poly A^+ RNA per lane from different tissues or cell lines. The blots were probed with a ^{32}P-labeled hHRI cDNA probe containing the open reading frame.

Results

cDNA array cDNA clones expressed in human dermal papilla were dot-blotted and used for hybridization. ^{32}P-dCTP labelled cDNA probes were prepared from the RNA samples isolated from normal ovary and ovarian cancer tissues, respectively. A total of 1,408 cDNA clones were compared by the cDNA array technique. Most of the dots exhibited identical intensity for both normal and ovarian cancer tissue. However, some genes showed striking differences in the levels of expression as indicated by the arrows in Fig. 1 and Table 1. One of the differentially expressed clones (K0631) was homologous to the heme-regulated inhibitor (HRI) of eIF-2α kinases.

Comparison of HRI expression between normal and cancerous human ovarian tissues The level of hHRI mRNA was analyzed in other matched ovarian samples by Northern blot hybridization to confirm its differential expression between normal and cancerous human ovarian tissues. hHRI was found to be down regulated in ovarian cancer tissues (Fig. 2). To check the equal RNA loading, the same blot was subsequently rehybridized with a β-actin gene probe after stripping (bottom panel).

Screening and analysis of HRI cDNA Northern data indicated that hHRI is about 3.0 kb in length but the clone K0631 which was used in the cDNA array was only 2.2 kb and contained only a partial open-reading frame. To obtain the full-length sequence of hHRI, we decided to screen the cDNA libraries. We screened the same dermal papilla cDNA library from which K0631 originated and the human prostate cDNA library. The positive cDNA clones were isolated using the K0631 clone as a probe. A total of 13 positive clones were isolated, which gave a 2,887 bp overlapping sequence, containing a full-length open reading frame. The

Table 1. Differentially expressed cDNA genes in normal and ovarian cancer cells.

EST number	Gene name
Elevated cDNA in normal ovarian cells	
B0007	Ubiquitin protease
B0191	Laminin-binding protein
K0631	Heme-regulated inhibitor
Elevated cDNA in ovarian cancer cells	
B0314	Gastrula-zinc finger protein
B0353	DNA-binding factor
B0606	PTP-IV1b (protein tyrosine phosphatase-IV1b)
B1021	CAP (adenyl cyclase-associated protein)
K0844	FIP-1 (adenovirus E3-14.7K interacting protein 1)
K1408	VBP-1 (VHL binding protein-1)
B1794	Novel
K1092	Novel
K1147	Novel
K1641	Novel

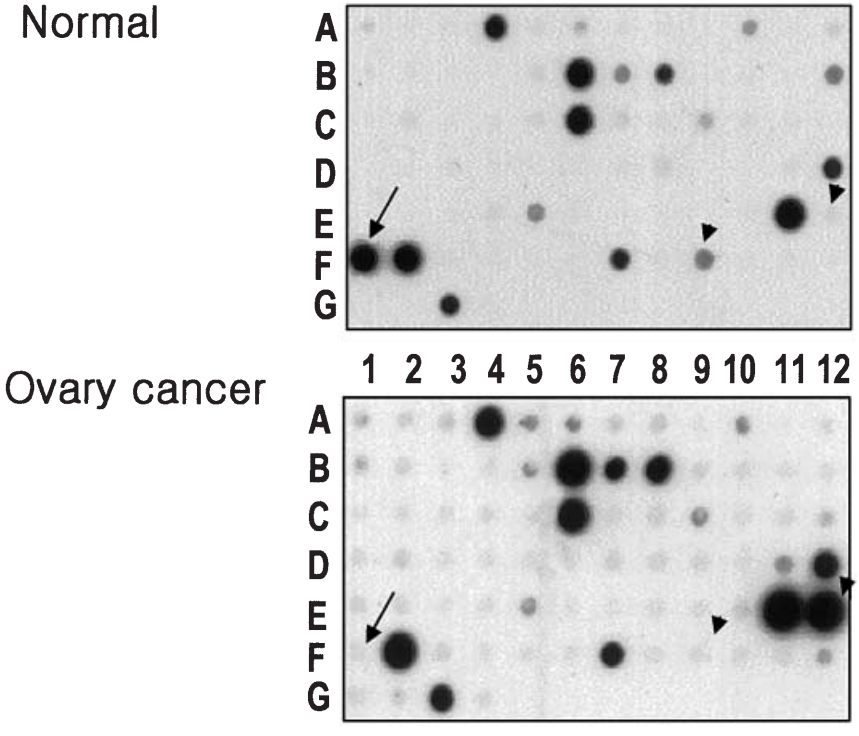

Fig. 1. cDNA array in epithelial ovarian cancer and normal ovarian tissue. A total of 1,408 cDNAs were arrayed on the positive charged nylon membranes and hybridized with ^{32}P-dCTP labelled probes. A total of 15 membranes were used in the cDNA array but only one membrane is shown here. F1, hHRI(K0631); E12, gastrula-zinc finger protein; F9, ubiquitin protease.

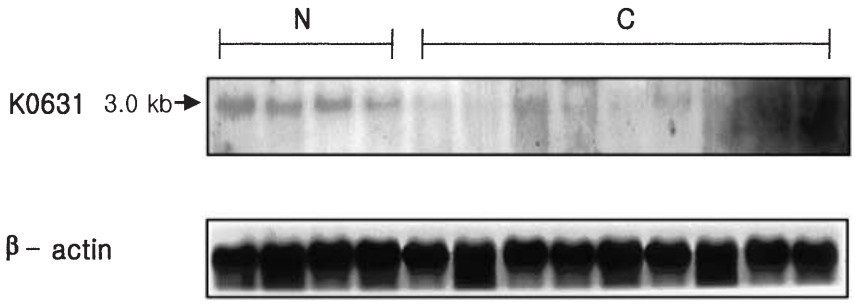

Fig. 2. Northern blot of hHRI shows that its expression is significantly decreased in epithelial ovarian carcinoma tissues, compared with normal ovarian tissues. N, normal ovary tissue; C, epithelial ovarian carcinoma tissue.

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  G TTA CTT CCG GGT CGG ACG GCG CTA GCT GCA GCA TCG GAG TGT GGC AGT GCT GGG CTG GCC GGC GGG CTG   70
GGC TGC GGC CGC GCG CGG CCG GCG ATG CAG GGG GGC AAC TCC GGG GTC CGC AAG CGC GAA GAG GAG GGC GAC  142
                                M  Q  G  G  N  S  G  V  R  K  R  E  E  E  G  D   16
GGG GCT GGG GCT GTG GCT GCG CGC CGG CAT CGA CTT TCC CGC CGA GGC CCG GAC CCC GAA TAT GAC GAA TCT  214
 G  A  G  A  V  A  A  R  R  H  R  L  S  R  R  G  P  D  P  E  Y  D  E  S   40
GAT GTT CCA GCA GAA ATC CAG GTG TTA AAA GAA CCC CTA CAA CAG CCA ACC TTC CCT TTT GCA GTT GCA AAC  286
 D  V  P  A  E  I  Q  V  L  K  E  P  L  Q  Q  P  T  F  P  F  A  V  A  N   64
CAA CTC TTG CTG GTT TCT TTG CTG GAG CAC TTG AGC CAC GTG CAT GAA CCA AAC CCA CTT CGT TCA AGA CAG  358
 Q  L  L  L  V  S  L  L  E  H  L  S  H  V  H  E  P  N  P  L  R  S  R  Q   88
GTG TTT AAG CTA CTT TGC CAG ACG TTT ATC AAA ATG GGG CTG CTG TCT TCT TTC ACT TGT AGT GAC GAG TTT  430
 V  F  K  L  L  C  Q  T  F  I  K  M  G  L  L  S  S  F  T  C  S  D  E  F  112
AGC TCA TTG AGA CTA CAT CAC AAC AGA GCT ATT ACT CAC TTA ATG AGG TCT GCT AAA GAG AGA GTT CGT AGG  502
 S  S  L  R  L  H  H  N  R  A  I  T  H  L  M  R  S  A  K  E  R  V  R  R  136
GAT CCT TGT GAG GAT ATT TCT CGT ATC CAG AAA ATC AGA TCA AGG GAA GTA GCC TTG GAA GCA CAA ACT TCA  574
 D  P  C  E  D  I  S  R  I  Q  K  I  R  S  R  E  V  A  L  E  A  Q  T  S  160
CGT TAC TTA AAT GAA TTT GAA GAA CTT GCC ATC TTA GGA AAA GGT GGA TAC GGA AGA GTA TAC AAG GTC AGG  646
 R  Y  L  N  E  F  E  E  L  A  I  L  G  K  G  G  Y  G  R  V  Y  K  V  R  184
AAT AAA TTA GAT GGT CAG TAT TAT GCA ATA AAA AAA ATC CTG ATT AAG GGT GCA ACT AAA ACA GTT TGC ATG  718
 N  K  L  D  G  Q  Y  Y  A  I  K  K  I  L  I  K  G  A  T  K  T  V  C  M  208
AAG GTC CTA CGG GAA GTG AAG GTG CTG GCA GGT CTT CAG CAC CCC AAT ATT GTT GGC TAT CAC ACC GCG TGG  790
 K  V  L  R  E  V  K  V  L  A  G  L  Q  H  P  N  I  V  G  Y  H  T  A  W  232
ATA GAA CAT GTT CAT GTG ATT CAG CCA CGA GCA GAC AGA GCT GCC ATT GAG TTG CCA TCT CTG GAA GTG CTC  862
 I  E  H  V  H  V  I  Q  P  R  A  D  R  A  A  I  E  L  P  S  L  E  V  L  256
TCC GAC CAG GAA GAG GAC AGA GAG CAA TGT GGT GTT AAA AAT GAT GAA AGT AGC AGC TCA TCC ATT ATC TTT  934
 S  D  Q  E  E  D  R  E  Q  C  G  V  K  N  D  E  S  S  S  S  I  I  F  280
GCT GAG CCC ACC CCA GAA AAA GAA AAA CGC TTT GGA GAA TCT GAC ACT GAA AAT CAG AAT AAC AAG TCG GTG 1006
 A  E  P  T  P  E  K  E  K  R  F  G  E  S  D  T  E  N  Q  N  N  K  S  V  304
AAG TAC ACC ACC AAT TTA GTC ATA AGA GAA TCT GGT GAA CTT GAG TCG ACC CTG GAG CTC CAG GAA AAT GGC 1078
 K  Y  T  T  N  L  V  I  R  E  S  G  E  L  E  S  T  L  E  L  Q  E  N  G  328
TTG GCT GGT TTG TCT GCC AGT TCA ATT GTG GAA CAG CAG CTG CCA CTC AGG CGT AAT TCC CAC CTA GAG GAG 1150
 L  A  G  L  S  A  S  S  I  V  E  Q  Q  L  P  L  R  R  N  S  H  L  E  E  352
AGT TTC ACA TCC ACC GAA GAA TCT TCC GAA GAA AAT GTC AAC TTT TTG GGT CAG ACA GAG GCA CAG TAC CAC 1222
 S  F  T  S  T  E  E  S  S  E  E  N  V  N  F  L  G  Q  T  E  A  Q  Y  H  376
CTG ATG CTG CAC ATC CAG ATG CAG CTG TGT GAG CTC TCG CTG TGG GAT TGG ATA GTC GAG AGA AAC AAG CGG 1294
 L  M  L  H  I  Q  M  Q  L  C  E  L  S  L  W  D  W  I  V  E  R  N  K  R  400
GGC CGG GAG TAT GTG GAC GAG TCT GCC TGT CCT TAT GTT ATG GCC AAT GTT GCA ACA AAA ATT TTT CAA GAA 1366
 G  R  E  Y  V  D  E  S  A  C  P  Y  V  M  A  N  V  A  T  K  I  F  Q  E  424
TTG GTA GAA GGT GTG TTT TAC ATA CAT AAC ATG GGA ATT GTG CAC CGA GAT CTG AAG CCA AGA AAT ATT TTT 1438
 L  V  E  G  V  F  Y  I  H  N  M  G  I  V  H  R  D  L  K  P  R  N  I  F  448
CTT CAT GGC CCT GAT CAG CAA GTA AAA ATA GGA GAC TTT GGT CTG GCC TGC ACA GAC ATC CTA CAG AAG AAC 1510
 L  H  G  P  D  Q  Q  V  K  I  G  D  F  G  L  A  C  T  D  I  L  Q  K  N  472
ACA GAC TGG ACC AAC AGA AAC GGG AAG AGA ACA CCA ACA CAT ACG TCC AGA GTG GGT ACT TGT CTG TAC GCT 1582
 T  D  W  T  N  R  N  G  K  R  T  P  T  H  T  S  R  V  G  T  C  L  Y  A  496
TCA CCC GAA CAG TTG GAA GGA TCT GAG TAT GAT GCC AAG TCA GAT ATG TAC AGC TTG GGT GTG GTC CTG CTA 1654
 S  P  E  Q  L  E  G  S  E  Y  D  A  K  S  D  M  Y  S  L  G  V  V  L  L  520
GAG CTC TTT CAG CCG TTT GGA ACA GAA ATG GAG CGA GCA GAA GTT CTA ACA GGT TTA AGA ACT GGT CAG TTG 1726
 E  L  F  Q  P  F  G  T  E  M  E  R  A  E  V  L  T  G  L  R  T  G  Q  L  544
CCG GAA TCC CTC CGT AAA AGG TGT CCA GTG CAA GCC AAG TAT ATC CAG CAC TTA ACG AGA AGG AAC TCA TCG 1798
 P  E  S  L  R  K  R  C  P  V  Q  A  K  Y  I  Q  H  L  T  R  R  N  S  S  568
CAG AGA CCA TCT GCC ATT CAG CTG CTG CAG AGT GAA CTT TTC CAA AAT TCT GGA AAT GTT AAC CTC ACC CTA 1870
 Q  R  P  S  A  I  Q  L  L  Q  S  E  L  F  Q  N  S  G  N  V  N  L  T  L  592
CAG ATG AAG ATA ATA GAG CAA GAA AAA GAA ATT GCA GAA CTA AAG AAG CAG CTA AAC CTC CTT TCT CAA GAC 1942
 Q  M  K  I  I  E  Q  E  K  E  I  A  E  L  K  K  Q  L  N  L  L  S  Q  D  616
AAA GGG GTG AGG GAT GAC GGA AAG GAT GGG GGC GTG GGA TGA AAG TGG ACT TAA CTT TTA AGG TAG TTA ACT 2014
 K  G  V  R  D  D  G  K  D  G  G  V  G  *                                    629
GGA ATG TAA ATT TTT AAT CTT TAT TAG GGT ATA GTT GGT ACA ATG CTT CGT TGT ATT TAG TAA GCC TTT ACA 2086
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GCT CCC TGC CCT CGT CAC TGC CCA AGC CCG GGC CTG CAC ATA CTC ACT GGA CTG TTC CAG TTT TGA CAG CTG 2374
CCA GTC TTC CTG CCC CTT TCA CAC TGC AGC TGA AGT TCA TTA CCT GAA GGA CGC CTC ATC ATT TCA TTC CTT 2446
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GCA CCC CAG ATG ATC TCC AGG CAG CTC AGA TCT CTT TCC TGC CTT TGC CCT GCA CTG TTC CCC GGT ACT TCC 2662
TCC TTT ATT GTA GCA CTC AGC TCC CCA GCC AAT CTG TAC ATC CCT CAG AGG CAG CGA TCT GAT GAA TTG GTT 2734
TTT GAA TCC CAG AAA GGG TCT GCC ATG GAG TTG GCA GTC ATC ACG GTA GAT GGC GTA TGA TTT TGC TGA ATT 2806
TTA AAT AAA ATG AAA ACC ATA AAT TAC ATG ATG CTT TTA TTG ACA CTT GAC AAC TGG CCT AAA TAA AAA GAC 2878
TCT GAC TCT AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA                            2887
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Fig. 3. The nucleotide sequence and deduced amino acid sequence of the hHRI gene. Amino acids are numbered from the initiating methionine. The stop codon is indicated by an asterisk. A polyadenylation signal is underlined. The two heme regulatory motifs (HRM1, HRM2) are shaded. The sequence has been deposited in *GenBank* (accession number AF 255050).

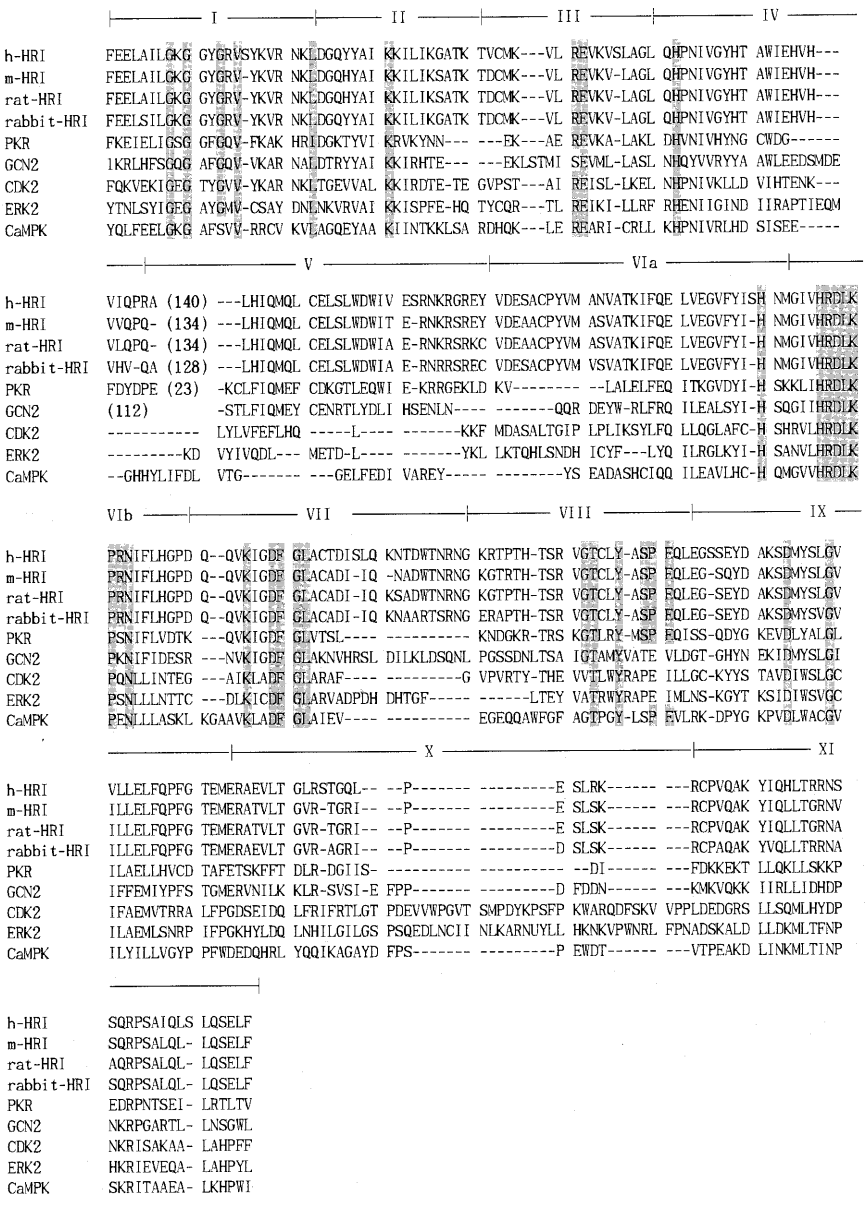

Fig. 4. Alignment of the conserved catalytic domains of hHRI with other serine/threonine protein kinases. The conserved catalytic domains are indicated by the Roman numerals (I → XI). The catalytic domains and subdomains of several kinases were aligned by using the DIALIGN2 program. There is an insertion of about 140 amino acids in HRI between domains IV and V as indicated. The consensus sequences are shaded.

nucleotide sequence analysis revealed that it has 94 nucleotides preceding the first ATG codon and then an open reading frame of 1,890 bp (nucleotides 95–1,981), followed by 803 bp of a 3′-noncoding region. The cDNA sequence was predicted to encode a protein of 630 amino acids (Fig. 3) with a molecular mass of 73 kDa. The amino acid sequence deduced from hHRI contains 12 conserved catalytic subdomains of Ser/Thr protein kinases (Fig. 4) and it posseses two conserved heme regulatory motifs (Fig. 3). Comparison of the amino acid sequences of hHRI with those of mouse liver, rat brain and rabbit reticulocyte HRIs displayed 85% overall amino acid sequence identities, respectively. It is notable that the greatest variation is concentrated in the unique insertion sequence of about 140 amino acids located between catalytic subdomains IV and V (Fig. 4). All these data indicated that the hHRI cDNA encodes a novel HRI family member. The motif analysis by the Prosite database showed that hHRI has several potential casein kinase-II phosphorylation sites, protein kinase C phosphorylation sites, and cAMP- and cGMP-dependent protein kinase phosphorylation sites.

Tissue distribution of hHRI To examine the tissue distribution and the level of expression of hHRI we performed Northern blot hybridization. Of the various

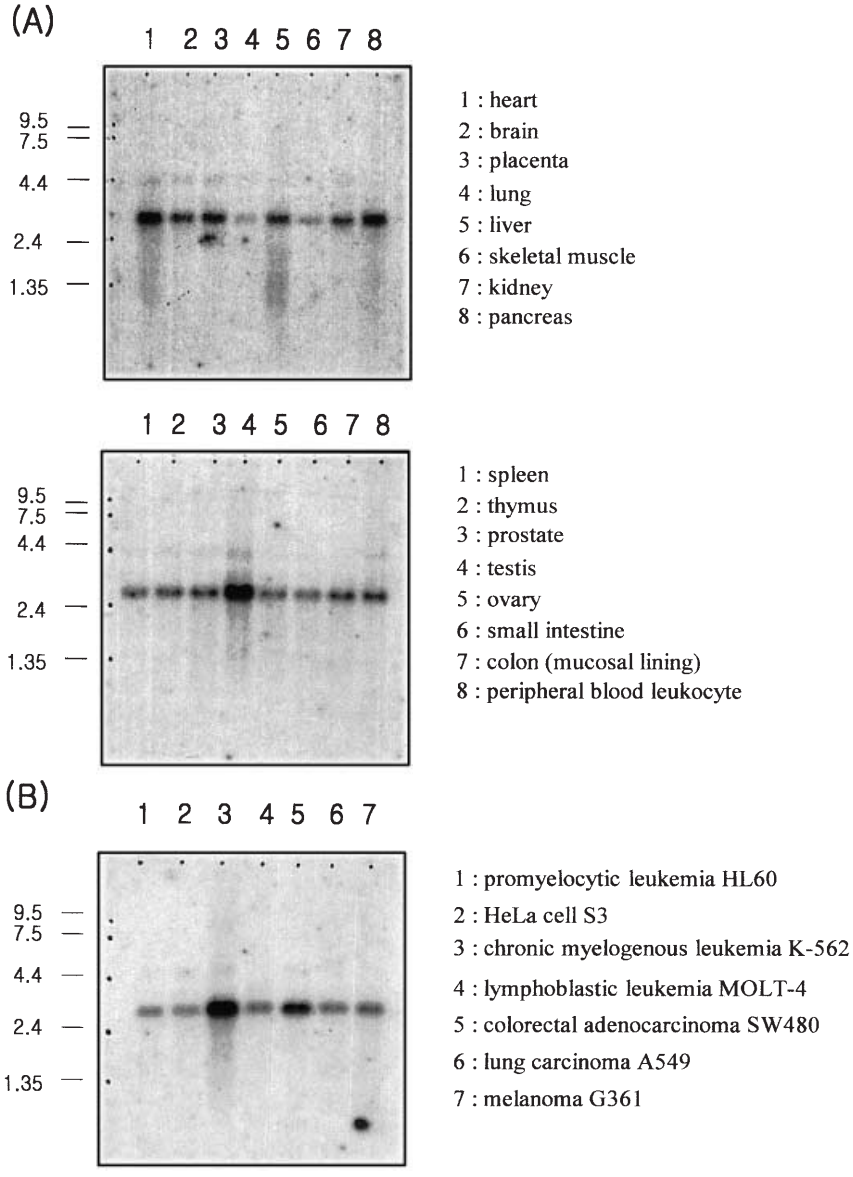

Fig. 5. Expression pattern of hHRI in normal human tissues and cancer cell lines. The blots with 2 μg of mRNA from several selected adult human tissues (**A**) and cancer cell lines (**B**) were hybridized with ^{32}P-dCTP-labelled hHRI cDNA. The positions of the RNA size markers are indicated in kilobases.

human tissues analyzed (Fig. 5A), hHRI transcripts were detected as a 3.0 kb single band. hHRI was ubiquitously expressed in a variety of human tissues and the level of transcription was relatively high in heart, testis, and pancreas and relatively low in lung, and skeletal muscle. In the cancer cell lines, the hHRI was found to be more abundantly expressed in chronic myelogenous leukemia K-562, and colorectal adenocarcinoma SW480 (Fig. 5B).

Discussion

We have cloned and sequenced a human homologue of HRI from a human dermal papilla cell. The molecular cloning of hHRI reveals an open reading frame that contains conserved catalytic domains of protein kinases and the consensus sequences of serine/threonine protein kinase (Fig. 4). hHRI cDNA contains all 12 catalytic domains with invariant amino acid residues. The consensus ATP-binding sequence Gly-Xaa-Gly-Xaa-Xaa-Gly and the invariant valine residue located two positions downstream from the Gly-Xaa-Gly-Xaa-Xaa-Gly are conserved in hHRI domain I. In domain II, the invariant lysine residue has been shown to be indispensable and to be involved in the phosphotransferase activity of protein kinases (Hanks and Hunter, 1995). In hHRI this invariant residue is Lys-196. Domain VI contains the consensus sequence that specifies either

serine/threonine protein kinase or tyrosine protein kinase. hHRI possesses Asp-Leu-Lys-Pro-Arg-Asn in domain VI, which is characteristic of the serine/threonine protein kinases. Asp-Phe-Gly located in domain VII is the most conserved short stretch in the catalytic domains of protein kinases and is probably involved in ATP binding. This motif is also found in hHRI as Asp-Phe-Gly in domain VII. In domain VIII the Ala/Ser-Pro-Glu consensus sequence essential for catalytic activity of the protein kinases is also found in HRI. Domain VIII of HRI contains the other consensus sequence for the serine/threonine protein kinases, Gly-Thr-Cys-Leu-Tyr, where underlined residues are conserved amino acids. The conserved amino acids in domain IX, also found in HRI, are likely to be involved in eIF-2 binding. This suggests that HRI cDNA encodes a serine/threonine protein kinase. HRI cDNA contains a unique insertional sequence of about 140 amino acids between catalytic domains IV and V. PKR and GCN2 also possess such inserts in subdomain V, but they are shorter than the HRI.

Hemin may inhibit the binding of ATP to HRI by inducing a conformational change in HRI that brings the essential sulfhydryl groups into close proximity and consequently promotes disulfide-bond formation. Formation of a disulfide-linked dimer is not unique to HRI, and has been reported for two receptor tyrosine kinases (RTKs), the colony-stimulating factor-1 (CSF-1) receptor and the platelet-derived growth factor (PDGF) receptor. Activation of all RTKs appears to involve receptor dimerization. The binding of CSF-1 (Li and Stanley *et al*., 1991) and PDGF (Li and Schlessinger *et al*., 1991) to their respective receptors stimulates both noncovalent dimerization and disulfide-linked dimerization of the receptors. The disulfide-linked dimerization of the CSF-1 receptor is thought to be involved in ligand-induced receptor internalization (Li and Stanley *et al*., 1991), but the exact role remains to be determined for both PDGF and CSF-1 RTKs. It is interesting to note that HRI and these two RTKs possess kinase insertion sequences that separate the conserved kinase domains. The kinase insertion sequences may be involved in dimerization.

In our study for the screening of differentially expressed genes in ovarian cancer using a cDNA array, we identified 13 differentially expressed genes (Table 1). Among them, only two genes, laminin-binding protein (van den Brule *et al*., 1994) and PTP family members (Warabi *et al*., 2000; Wiener *et al*., 1996), were previously reported that were up- or down-regulated in ovarian cancers. The remaining 11 genes need to be investigated.

We have provided evidence that the mRNA encoding hHRI is expressed in all human tissues examined by Northern analysis and revealed a broad range of expression levels of hHRI mRNA in many of the tissues investigated. The general abundance of hHRI mRNA in human nonerythroid tissues may indicate that HRI plays an additional role in mammalian cells. It will be important to establish alternative mechanisms of regulation of HRI in those cells that do not produce significant levels of hemin.

Overexpression of the dominant-negative PKR mutants (Lengyel, 1993) or of the 58 kDa cellular inhibitor of PKR (Barber *et al*., 1994) in NIH 3T3 cells has been shown to cause oncogenic transformation. Thus, eIF-2α kinases may be considered as tumor suppressors. The down regulation of hHRI in epithelial ovarian cancer also suggested that it may work as an important regulator of tumor cell metabolism. So far, eIF-2α is the only known physiological substrate of HRI, but it has been reported that HRI and PKR can phosphorylate the inhibitor of a ubiquitous nuclear transcriptional factor, NF-κB (Ghosh and Baltimore, 1990; Kumar *et al*., 1994). Thus, our finding that hHRI, like PKR, is an ubiquitous mammalian eIF2α kinase suggests that these proteins may also play a role in the growth and differentiation of nonerythroid cells.

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