Purification of Human Tumor Cell Autocrine Motility Factor and Molecular Cloning of Its Receptor*

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Tumor autocrine motility factor (AMF) has been detected in and purified from serum-free conditioned medium of human HT-1080 fibrosarcoma cells. Under nonreducing conditions, AMF migrates in sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a single band of 55 kDa but under reducing conditions as a band of 64 kDa. Two-dimensional polyacrylamide gel electrophoresis of the purified AMF resolved two groups of polypeptides with isoelectric points of 6.1 and 6.2 (majors), 6.35 and 6.4 (minors). Purified AMF stimulated HT-1080 cell migration in a dose-dependent fashion. The motility stimulation of the fibrosarcoma cells with AMF is associated with the phosphorylation of the AMF receptor, a 78-kDa cell surface glycoprotein (gp78), suggesting protein kinase participation in migratory signal transduction. The gene encoding gp78 was cloned from an HT-1080 fibrosarcoma complementary DNA library. The deduced sequence encodes a polypeptide of 323 amino acids. The nucleotide and predicted amino acid sequence of the gp78 reveals significant homology with the human suppressor/oncogene p53 protein.

Kinesis of eukaryotic cells plays a fundamental role in diverse biological phenomena such as spermatogenesis, morphogenesis, wound healing, chemotaxis, and metastasis (1). However, despite many studies the mechanisms underlying cell movement and motility stimulation are not fully understood. Recently a group of cytokines has been described which are distinguished by their ability to stimulate motility in a variety of eukaryotic cells. The specificity of their expression and action may be a mechanism by which cellular motility is controlled and regulated within the organism. A fibroblast-derived scatter factor has been shown to induce the dispersion of Madin-Darby canine kidney epithelial cells and to induce invasiveness of epithelial tumor cells (2–4). Migration stimulating factor is produced by human fetal and tumor-derived fibroblasts and enhances cell migration into collagen gel matrices (5, 6). AMF is produced by and enhances the motility of the producing cells (7, 8). These factors and others may represent a family of cytokines whose regulated expression induces cell motility in normal situations such as wound healing (scatter factor) and embryogenesis (migration stimulating factor) and whose constitutive autocrine expression (AMF) may confer metastatic capabilities on neoplastic cells. AMF-stimulated tumor motility is coupled to pseudopodial protrusion (9), with an increased rate of phospholipid methylation (7), and it was suggested that AMF initiates cell motility by interacting with a cell surface receptor that is coupled to a pertussis toxin-sensitive G protein (10). We have demonstrated previously that a monoclonal antibody (mAb) against a 78-kDa B16-F1 melanoma cell surface glycoprotein (gp78) and B16-F1 melanoma serum-free medium containing AMF stimulate cell motility through a GTP-binding protein pathway and designated gp78 as the receptor for B16-F1 AMF (11). AMF was purified previously from the human A2058 (7) and from the mouse B16-F1 melanoma serum-free conditioned media. In both preparations AMF migrates under nonreducing conditions in SDS-PAGE as a single band of 55 kDa whereas under reducing conditions it migrates as a single polypeptide of 64 kDa. Purified AMF stimulates B16-F1 cell migration in a dose-dependent fashion and binds directly in a protein-protein binding assay to the gp78-AMFR receptor.

In this paper we demonstrate that the 55-kDa (nonreduced) and 64-kDa (reduced) forms of AMF are not melanoma unique as a similar AMF molecule was purified from the human HT-1080 fibrosarcoma serum-free culture conditioned medium, and the present data show that the motility stimulation of HT-1080 cells with AMF is associated with gp78 phosphorylation. Furthermore, here we report on the molecular cloning of the human gp78-AMFR. Unexpectedly, the nucleotide and predicted amino acid sequences of this clone reveal significant homology with the human p53 protein, and this is the only polypeptide found to date to be homologous to p53.

EXPERIMENTAL PROCEDURES

Cells and Culture Conditions—The cell lines used in this study were the human HT-1080 fibrosarcoma (obtained from the American Type Culture Collection [ATCCCATL21]), the mouse B16-F1 mela-

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1 The abbreviations used are: AMF, autocrine motility factor; gp78, glycoprotein of 78 kDa; AMFR, autocrine motility factor receptor; mAb, monoclonal antibody; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CMF, Ca2+—and Mg2+—free; PBS, phosphate-buffered saline (pH 7.2); BES, 2-(N-hydroxyethyl)aminomethanesulfonic acid.
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Molecular Cloning of the Human gp78—A human cervical carcinoma, HeLa cells agt11 cDNA library (provided by Dr. J. Christman, Michigan Cancer Foundation, Detroit) was screened at a density of 15,000 plaque-forming units/150-mm plate, according to Young and Davis (19) as described (20). Briefly, after 3 h at 42 °C the plates were transferred to 37 °C, and nitrocellulose filters were presaturated with 10 mM isopropl 1-thio-β-galactopyranoside were overlaid on the agar for 16 h. After washing with TBS buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8, containing 10% low fat milk and 0.05% sodium azide) the filters were incubated with rat anti-gp78 antibody (11), preabsorbed with lysate Escherichia coli strain Y1088 bound to nitrocellulose and diluted 1:500 in TBS buffer for 2 h at 24 °C. After washing in TBS buffer containing 0.02% Tween 20, the filters were reincubated with secondary antibody (goat anti-rat Ig) for 30 min (21) at 37 °C with agitation, washed extensively, as above. The filters containing bacteriophages that generated positive signals were processed through successive rounds of antibody screening until 100% positive plaques were obtained. One of the clones, HL6, was processed as follows. Plaque-purified phages were incubated in LB medium with E. coli Y1088, grown for 4 h at 42 °C, and induced by isopropl 1-thio-β-galactopyranoside to a final concentration of 10 mM at 37 °C for 3 h. Fused protein was extracted by adding 0.01 volume of 10 × extraction buffer (10 mM NaHPO4, pH 7.5, 68 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 0.4 mM phenylmethylsulfonyl fluoride). Extracted fused proteins were separated by 8% SDS-PAGE and transferred to a nitrocellulose filter. The filter was incubated with mouse anti-β-galactosidase antibody (Promega Biotec, Madison, WI) or the anti-gp78 mAb (11). Positive bands were identified with [125]I-labeled sheep anti-rat Ig or [125]labeled goat anti-mouse Ig (Amersham Corp.) for gp78 or β-galactosidase, respectively. The HL6 cDNA clone was 5' end-labeled with [32P]dATP and used as a probe to screen 1.6 × 10^7 recombinant phages from a human HT-1080 fibrosarcoma cDNA library (Clontech). Briefly, plaques were transferred to nitrocellulose and were prehybridized at 65 °C for 3 h in a solution containing 5 × Denhardt's solution (6 × SSC, 0.1% SDS, 0.1 M NaPO4, pH 7.0), and 150 μg/ml denatured herring sperm DNA. Hybridization was done overnight at 65 °C in prehybridization solution containing 10^-4 cm^3/ml probe. After hybridization, filters were washed once in 3 × SSC, 0.1% SDS for 30 min, once in 0.5 × SSC, 0.1% SDS for 30 min, and once in 0.1% SDS for 15 min. All washes were at 65 °C. cDNA restriction fragments were subcloned into PGEM-3Zf(-) (Promega) vector, and the nucleotide sequence of the cDNA was determined in both strands, in opposite directions by the dideoxy chain termination method. The T7 DNA polymerase Sequenase version 2.0 system was used for sequencing according to the instructions of the manufacturer (U.S. Biochemical Corp.).

Northern Blot Analysis—Five μg of poly(A)+ RNA was fractionated by electrophoresis on 1% formaldehyde agarose gels and blotted onto nitrocellulose. The filters were probed with [32P]dATP- and [32P]dCTP-labeled nick-translated probes (specific activity, 2–5 × 10^6 cpm/μg; 3 × 10^5 cpm/filter). The filters were washed twice in 2 × SSC, 0.2% SDS for 30 min at room temperature and twice in 0.1 × SSC, 0.1% SDS for 1 h at 37 °C. The blots were exposed to X-ray film and the autoradiographs were developed. After autoradiography, the membranes were rehybridized to a final concentration of 10^-4 cm^3/ml probe. After hybridization, filters were washed once in 3 × SSC, 0.1% SDS for 30 min, once in 0.5 × SSC, 0.1% SDS for 30 min, and once in 0.1% SDS for 15 min. All washes were at 65 °C. cDNA restriction fragments were subcloned into PGEM-3Zf(-) (Promega) vector, and the nucleotide sequence of the cDNA was determined in both strands, in opposite directions by the dideoxy chain termination method. The T7 DNA polymerase Sequenase version 2.0 system was used for sequencing according to the instructions of the manufacturer (U.S. Biochemical Corp.).

RESULTS

Purification of HT-1080 AMF—Tumor AMF is a cytokine that stimulates both random and directed migration by self-producing cells (7, 8, 11). HT-1080, a human fibrosarcoma cell line, was utilized for these studies because it was found to secrete into the culture medium potent AMF activity. For AMF purification, serum-free conditioned medium was concentrated and subjected to molecular sieve chromatography. Each fraction was analyzed for its effect on HT-1080 cell motility by the phagokinetic track motility assay; the activity...
was found to be eluted as a single peak as found for B16-F1 AMF. This material was collected, iodinated, and separated by 12.5% SDS-PAGE in the presence or absence of mercaptoethanol and found by autoradiography to comprise a single polypeptide (Fig. 1). Under reducing conditions the band migrates with an apparent molecular mass of 64 kDa and under nonreducing conditions as 55 kDa, suggesting that it is rich in cysteines forming intrapeptide disulfide bonds. Similar molecular sizes (under reducing and nonreducing conditions) were reported previously for the human A2058 and the mouse B16-F1 melanoma AMFs (7), implying that the human and the mouse AMFs are likely to be similar and that the 55-kDa AMF is not melanoma specific.

The purified HT-1080 AMF stimulates HT-1080 cell motility in a dose-dependent fashion and exerts maximal stimulating activity between 10 and 20 pg/ml (Fig. 2A). Fig. 2, B and C, is a pictorial demonstration of the motility assay and the stimulatory effect of the AMF. HT-1080 cells were plated on gold particle-coated substrate in the absence (Fig. 2B) or presence (Fig. 2C) of 10 pg/ml AMF. The AMF stimulates cell motility from 8.2 ± 1.3 μ²/h (Fig. 2B) to 17.8 ± 1.5 μ²/h (Fig. 2C). The 2-fold stimulation of the HT-1080 AMF is similar in magnitude to the effect of the A2058 and the B16-F1 AMFs on their respective cells (7, 11).

To test whether the 55-kDa AMF band is comprised of one or more polypeptides, the 125I-labeled AMF was subjected to two-dimensional PAGE. The 125I-labeled AMF resolved into two groups of polypeptides exhibiting pl values of 6.1, 6.2 (major) and 6.35, 6.4 (minor), respectively (Fig. 3). This observation raised the possibility that the HT-1080 AMF is a glycoprotein and that subtle changes in the carbohydrate side chain composition may be responsible for the appearance of the four polypeptides with different isoelectric focusing points. Treatment of the 125I-labeled AMF with neuraminidase (0.1 units for 30 min at 37 °C) to remove terminal sialic acid residues did not affect its apparent molecular weight on SDS-PAGE (not shown). In addition, a nonradioactive AMF was separated by SDS-PAGE, electroblotted to nitrocellulose, and subjected to a 125I-labeled wheat germ agglutinin overlay procedure (21). No radioactive band was detected after prolonged (2 weeks) autoradiography (not shown). Based on these two criteria we have concluded that HT-1080 AMF is most probably not a glycoprotein. The AMF polypeptide lost its migratory stimulation activity after heat inactivation (1 min at 100 °C) and/or reduction with 2 mM mercaptoethanol.

Demonstration That Human Tumor Cells Express a Phosphorylated gp78—We have shown recently that a 78-kDa surface glycoprotein (gp78) of murine B16-F1 melanoma cells is involved in the stimulation of cell motility in vitro and metastasis in vivo (11, 21). We have designated gp78 as the B16-F1 melanoma AMF receptor (AMFR). In later studies gp78 was found not to exhibit melanoma specificity, as it is both present on and mediates cell migration of other cells (22, 23). Immuno precipitation of [35S]cysteine-labeled cells was performed to establish the identity of the human target antigen reactive with mouse anti-gp78 antibodies. As shown in Fig. 4, a rat mAb raised against affinity-purified mouse gp78 (11) recognizes its antigen out of total cell extract proteins.
The migration of 78 kDa proteins were analyzed by 8% SDS-PAGE and visualized by fluorography. The phosphoproteins were analyzed by 8% SDS-PAGE and visualized by autoradiography. Lane M, 12C-protein markers from top, 92.5, 69, 46, and 30 kDa.

FIG. 4. Immunoprecipitation analysis of tumor cell extracts by anti-gp78 mAb. a, B16-F1 mouse melanoma; b, HT-1080 human fibrosarcoma; c, S3-HeLa cervical carcinoma. a’, b’, and c’, control immunoprecipitations with preimmune serum. Arrow at left points to the migration of 78 kDa. Subconfluent cultures were washed twice with PBS and incubated for 1 h in L-cysteine-free Eagle’s medium supplemented with 5% dialyzed fetal bovine serum. L-[35S]Cysteine (100 μCi/ml) was added, and the cultures were reincubated for an additional 4 h at 37 °C. Cell extraction and immunoprecipitation were performed as described under “Experimental Procedures.” The proteins were analyzed by 8% SDS-PAGE and visualized by fluorography. Lane M, 12C-protein markers.

FIG. 5. Phosphorylation of gp78 from resting and migration-stimulated HT-1080 fibrosarcoma cells. Resting HT-1080 cells (a and b) and AMF-stimulated cells (c and d) were labeled with 32P and immunoprecipitated with preimmune serum (a and c) or anti-gp78 mAb (b and d) (for details see “Experimental Procedures.” The phosphoproteins were analyzed by 8% SDS-PAGE and visualized by autoradiography. Lane M, 12C-protein markers.

from B16-F1 cells and from two human cell lines, i.e. HT-1080 fibrosarcoma and S3-HeLa cervical carcinoma (Fig. 4).

It was shown previously that the motility stimulation pathway of AMF and of anti-gp78 mAb, which mimics the physiological effect of AMF, involves a GTP-binding protein(s) (7, 11). To study further the role of gp78 in AMF motility stimulation, gp78 was examined by labeling HT-1080 cells and AMF-stimulated cells (c and d) were labeled with 32P and immunoprecipitated with preimmune serum (a and c) or anti-gp78 mAb (b and d) (for details see “Experimental Procedures.” The phosphoproteins were analyzed by 8% SDS-PAGE and visualized by autoradiography. Lane M, 12C-protein markers.

The isolated gp78-hAMFR cDNA was used as a probe to analyze the mRNA expressed in human tumor cell lines (Fig. 6). A single size transcript of ~ 3.5 kilobases was detected in both S3-HeLa and HT-1080 fibrosarcoma cells. The 1.9 kilobases of hAMFR cDNA were subcloned and sequenced from both ends (Fig. 7). The HL6 insert was sequenced in parallel, and the two cDNA clones were found to be identical over the overlapping regions that encompassed the first 490 base pairs of the 5’ end. Fig. 7 depicts the nucleotide sequence of the 176-base 5’-flanking region which is high in G + C content (62%), 969 coding, and 665 3’-flanking nucleotides including the consensus polyadenylation signal (AATAAA). The discrepancy between the size of the isolated cDNA clone and that of the transcript estimated from Northern analysis may be a result of a deficiency of 5′ sequence, lack of poly(A) tail, or both. The nucleotide sequence of gp78 contains an open reading frame encoding for 323 amino acids with an initiating methionine at nucleotide 177 (Fig. 7). The mature protein is predicted to begin at Ala-18 which follows the putative leader peptide with a peptide cleavage signal site that obeys the (-3,-1) rule (24). The gp78-hMFR has the structural features of an integral membrane protein, with a hydrophobic stretch of 25 amino acids located between residues 111 and 137, consistent with a transmembrane segment that makes a single helical span. The extracellular NH2 terminus includes one potential N-linked and several potential O'-linked glycosylation sites, supporting the previous finding that gp78 is glycosylated with both N- and O’-linked oligosaccharides (21).

A computer-assisted search of data bases for gene homology (data bases from GenBank, European Molecular Biology Laboratory, and the National Biomedical Research Foundation were analyzed with software from the University of Wisconsin Genetics Computer Group and the WORDSEARCH, ALIGN and FASTA programs, last test screen, December 6, 1990) revealed that the gp78-hAMFR cDNA has not been cloned previously. Surprisingly, however, the gp78-hMFR cDNA nucleotide sequence was found to share 50.1% homology with the human p53 cDNA nucleotide sequence (not shown). Optimal alignment of the deduced amino acid sequences of the

FIG. 6. RNA blot analysis of gp78-hMFR a, HT-1080 human fibrosarcoma and b, S3-HeLa human cervical carcinoma. Positions of 18 and 28 S rRNA are indicated at the left. Five μg of polyadenylated RNA was fractionated by electrophoresis on 1% formaldehyde-agarose gels and blotted onto nitrocellulose. The filters were probed with 32P-dATP- and 33P-dCTP-labeled nick-translated gp78-hMFR (specific activity, 2–5 x 106 cpm/μg; 3 x 107 cpm/filter). The filters were washed twice in 2 x SSC, 0.2% SDS for 30 min at room temperature and twice in 0.1 x SSC, 0.1% SDS for 30 min at 50 °C and visualized by autoradiography.
two cDNA clones (ALIGN program) revealed 27.2% identity over 296 amino acid residues, and when conserved amino acid substitutes are considered the degree of homology increases to 44.5% (Fig. 8).

**DISCUSSION**

The data presented in this report establish that HT-1080 fibrosarcoma cells secrete an AMF with an apparent molecular mass of 55 kDa on SDS-PAGE. Under reducing conditions, AMF exhibits an apparent molecular mass of 64 kDa. The purified AMF induces motility of the secreting HT-1080 cells in a dose-dependent fashion. AMFs have been purified from A2058 human and B16-F1 mouse melanoma conditioned media and migrated on non-reduced and reduced SDS-PAGE with molecular weights identical to the HT-1080 AMF (7, Fig. 1). It was proposed that the higher migration of AMF in the presence of a reducing agent indicates the reduction of existing intrachain disulfide bonds. The HT-1080 fibrosarcoma AMF was resolved by isoelectric focusing into two groups of four polypeptides with pI values identical to the minor human AMF (7, Fig. 1). It was proposed that the higher migration of AMF in the presence of a reducing agent indicates the reduction of existing intrachain disulfide bonds. The HT-1080 fibrosarcoma AMF was resolved by isoelectric focusing into two groups of four polypeptides with pI values identical to the minor human AMF (7, Fig. 1).

AMFs are expressed from the same gene coding for AMFs of the same molecular weight or that are covalently bound carbohydrate in purified HT-1080 AMF. Under reducing conditions, AMF exhibits an apparent molecular mass of 64 kDa. The purified AMF induces motility of the secreting HT-1080 cells in a dose-dependent fashion. AMFs have been purified from A2058 human and B16-F1 mouse melanoma conditioned media and migrated on non-reduced and reduced SDS-PAGE with molecular weights identical to the HT-1080 AMF (7, Fig. 1). It was proposed that the higher migration of AMF in the presence of a reducing agent indicates the reduction of existing intrachain disulfide bonds. The HT-1080 fibrosarcoma AMF was resolved by isoelectric focusing into two groups of four polypeptides with pI values identical to the minor human AMF (7, Fig. 1).

**Fig. 7.** The cDNA sequence and predicted amino acid sequence of gp78-hAMFR. Nucleotides are numbered positively in the 5' to 3' direction, beginning with the initiator methionine and are numbered negatively in the 3' to 5' direction (right). The predicted amino acid sequence is shown below the nucleotide sequence in one-letter amino acids (left). The transmembrane domain is singly underlined, the potential N-linked glycosylation site is doubly underlined, and cysteines are identified by asterisks.

**Fig. 8.** Homology between the gp78-hAMFR and the human p53 protein. Identical residues are boxed. Dashes indicate gaps for optimal alignment.
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factors (7, 28). The binding of anti-gp78 mAb to its antigen was inhibited (10-fold) by preincubation with B16-F1 AMF-containing serum-free conditioned medium. Based on such functional properties it was suggested that gp78 is the AMF receptor of B16-F1 melanoma cells (11). More recently we showed that immunopurified gp78, immobilized on nitrocellulose, binds purified AMF directly in a protein-protein binding assay; this in conjunction with the competitive inhibition of AMF-stimulated cell motility by soluble gp78 has demonstrated that AMF is the natural ligand of gp78. In this report the human homologue of the murine gp78 was identified and found to be of identical molecular weight in SDS-PAGE.

The motility stimulating pathway of AMF and of a chemotactic peptide involves a GTP-binding protein (7, 11, 28). Here we show that gp78-hAMFR undergoes phosphorylation after interaction with its ligand, which adds a new insight into the poorly defined molecular pathway by which AMF manifests its effect on cells. The gp78-hAMFR cDNA contains the Ser/Thr-Xaa-Lys/Arg motif in the cytoplasmic (Ser-Gly-Lys 194–196) domain, which fits the consensus sequence for a phosphorylation site (29). The cytoplasmic domain of gp78-hAMFR also includes a nucleotide-binding consensus sequence (Gly-Xaa-Gly-Xaa-Xaa-Gly), which is found in nucleotide-binding proteins including serine/threonine kinases (30) and is located at the 157–162 amino acid domain. Thus, activation of gp78 may result from its autophosphorylation after ligand-binding or from a GTP-binding “coupling” protein(s) that is associated with the receptor on the cytoplasmic face of the membrane, as described for rhodopsin, β-adrenergic muscarinic acetylcholine receptors and the mating receptor of yeast (for review see Ref. 27). Alternatively gp78 may be coupled to a different integral membrane protein by a GTP-binding protein as described for the formyl-chemotactic peptide receptor (28). Most GTP-binding proteins are activated following GTP binding and become deactivated upon GTP hydrolysis. This may also provide a mechanism for coupling the energy of GTP hydrolysis to the motility process.

Gp78-hAMFR was cloned from a human fibrosarcoma cDNA library, and a cDNA clone containing the complete coding region was characterized. The sequence contains a single open reading frame encoding 323 amino acids with features typical of an integral membrane protein. A hydrophobic region of 25 amino acids is located between residues 111 and 137, consistent with a transmembrane segment that makes a single helical span. The sequences upstream of this element includes one potential N-linked and several potential O-linked glycosylation sites, supporting the previous finding that gp78 is glycosylated with both N- and O-linked oligosaccharides (21). Based on the above and on the facts that the antigenic recognition site of the anti-gp78 mAb AMF binding site on gp78 and the N-linked glycosylation site all reside on the extracellular exposed domain of gp78-hAMFR, we conclude that the NH2 terminus of gp78 is extracellularly exposed.

A computer search of several sequence data bases queried with the entire 1,810 base pairs of the gp78-hAMFR revealed the most surprising finding of this report: the homology of gp78 to the human p53 cDNA. p53 codes for a cellular serine-phosphoprotein, which normally acts as a tumor suppressor gene, whereas its mutated form acts as an oncogene. The gene product is thought to be involved in cell growth regulation and was shown to form protein-protein complexes mainly with viral antigens (for review see Ref. 12). The available data concerning the evolutionary consideration of p53 reveal that this gene is restricted to vertebrates. The gene encodes a group of molecules ranging in size from 362 to 396 amino acid residues with internal homology from 47.1% (human-Xenopus) to 95.7% (human-monkey). All of the p53 polypeptides share five highly conserved domains (I-V). Although it is evident that gp78-hAMFR is not a p53-like molecule since it lacks the five distinct conserved domains and is a membrane protein, there are several similar structural features between p53 and gp78 which permit the speculation that both genes may have diverged from a common ancestral gene, probably prior to the appearance of osteichthyes: 1) the nucleotide and amino acid sequence homology; 2) both are phosphoproteins; 3) both have one potential N-linked glycosylation site; 4) both have protein-binding sites; 5) the amino and carboxyl termini of both molecules are helical and hydrophobic whereas the internal amino acid sequence domain is hydrophilic and high in β-sheet. The last parameter is of special interest since it has been pointed out that structure is better conserved through evolution than sequence (12).

The availability of a cDNA clone containing the complete coding region for a human motility factor receptor will now permit detailed studies into the molecular mechanisms by which ligand-receptor interactions induce cell migration. Site-directed mutagenesis and expression of modified gp78 in mammalian cells will make it possible to study the biochemical mechanisms of gp78 function. In addition, these results may lead to a better understanding of the structure-function relationships of the p53 protein.

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