Crystal Structure of the Human Laminin Receptor Precursor*†‡

The human laminin receptor (LamR) interacts with many ligands, including laminin, prions, Sindbis virus, and the polyphenol (-)-epigallocatechin-3-gallate (EGCG), and has been implicated in a number of diseases. LamR is overexpressed on tumor cells, and targeting LamR elicits anti-cancer effects. Here, we report the crystal structure of human LamR, which provides insights into its function and should facilitate the design of novel therapeutics targeting LamR.

The human LamR precursor protein and the p40 ribosomal protein are encoded by the same gene (37LRP/p40) (1), demonstrating that human LamR is a protein that has acquired dual function through evolution, acting as both a cell surface receptor and a ribosomal protein (2). In addition to its role as a ribosomal protein, LamR is a nonintegrin cell surface protein that has been identified as the receptor for the extracellular matrix molecule laminin-1 (3), pathogenic prion protein (4), Sindbis virus (5), Venezuelan equine encephalitis virus (6), cytotoxic molecule laminin-1 (3), pathogenic prion protein (4), Sindbis virus (5), Venezuelan equine encephalitis virus (6), cytotoxic mitoribosomal protein, LamR is a nonintegrin cell surface protein that share 60% sequence identity (see Fig. 1). Rps0 is responsible for 20 S ribosomal RNA processing and maturation of the 40 S ribosomal protein subunit, directly affecting protein synthesis levels (13). Both the localization of human LamR to more than one intracellular component and the multiple functions of its homolog in yeast suggest that human LamR may also have numerous functions at the intracellular level.

EXPERIMENTAL PROCEDURES

Recombinant LamR Expression and Purification—Residues 1–220 of human 37-kDa LamR precursor protein (LamR220) were subcloned from a full-length LamR cDNA into an Escherichia coli expression vector that includes a tobacco etch virus-cleavable, N-terminal His6 tag. The construct was verified by automated DNA sequencing. The vector encoding LamR220 was transformed into E. coli strain BL21 (DE3*), and cultures were grown in Luria broth medium at 37 °C to an A600 of 0.6. Protein expression was induced by the addition of isopropyl-thiogalactopyranoside (0.1 mM) for 12 h at 20 °C. Cells were harvested, resuspended in lysis buffer (50 mM Tris (pH 8.0), 300 mM NaCl, 0.1% Triton X-100, 10% glycerol, EDTA-free protease inhibitor tablet (Roche Applied Science)), and lysed by French press. The lysate was centrifuged at 16,000 RPM for 30 min, and the supernatant was collected. The soluble fraction was purified by nickel-nitrioltriacetic acid chromatography (Qiagen) followed by gel filtration chromatography (Superdex 75, Amersham Biosciences). Protein was concentrated in spin concentrators (Amicon, Millipore).

Crystalization and Data Collection—Crystals of LamR220 were grown at 17 °C by the hanging-drop vapor diffusion method in drops containing a 1:1 (v/v) ratio of protein solution at 10 mg/ml and reservoir solution containing 17% (w/v) polyethylene glycol 10,000, 120 mM sodium citrate, 100 mM MES (pH 6.0), and 5% (w/v) polyethylene glycol 1,500. Crystals belong to the tetragonal space group P42_2_2 with unit cell dimensions of a = 75.69 Å, b = 75.69 Å, and c = 99.01 Å. Crystals were soaked briefly in crystallization buffer with 20% glycerol (v/v) and then flash-frozen in liquid nitrogen. There is one LamR220 molecule in the asymmetric unit with a solvent monomer (37 kDa) and dimer (67 kDa) (4). The homo- or heterodimeric state of 67-kDa LamR has yet to be resolved, but its association with the cell surface is mediated by fatty acid acylation (9). LamR expression acts as a prognostic factor in determining the degree of malignancy of human cancer patients (10). Overexpression of LamR correlates with a highly invasive cell phenotype and increased metastatic ability (10), mediated by the high affinity binding between LamR and laminin in the extracellular matrix. The dual function of human LamR as a receptor at the cell surface and as a component of the translational machinery may be important for understanding how overexpression of LamR in cancer affects disease pathogenesis.

The role of LamR as a ribosomal protein is also of significant interest. Intracellular LamR is localized on the 40 S ribosome (11) and in the nucleus (12). Human LamR and Rps0, the homolog in yeast, which does not exhibit laminin binding activity, share 60% sequence identity (see Fig. 1). Rps0 is responsible for 20 S ribosomal RNA processing and maturation of the 40 S ribosomal protein subunit, directly affecting protein synthesis levels (13). Both the localization of human LamR to more than one intracellular component and the multiple functions of its homolog in yeast suggest that human LamR may also have numerous functions at the intracellular level.
content of 56%. Diffraction data were collected to a resolution of 2.15 Å at X29 at the National Synchrotron Light Source at Brookhaven National Laboratory. Data were processed with HKL2000 (14). A molecular replacement solution was found with AMoRe (15) using as a search model the structure of the Archaeoglobus fulgidus 30 S ribosomal protein S2p (PDB code 1V16, chain A) (16). Rigid body refinement, simulated annealing, and positional and B-factor refinement were performed with CNS (17) and Refmac (18). Coot was used for model building (19). According to PROCHECK (20), 95.4% of the residues have backbone torsion angles in most favored regions, and 4.6% have backbone torsion angles in additional allowed regions.

In Vitro Binding Affinity for laminin-1—LamR220 and full-length LamR (LamR295) binding affinity for laminin-1 were tested in vitro. White polystyrene enzyme-linked immunosorbent assay 96-well microplates, precoated with murine laminin-1 (New England Biolabs), were blocked overnight at 4 °C with blocking buffer (3.0% BSA, 0.1% sodium azide in phosphate-buffered saline). Wells were incubated with increasing concentrations of LamR220 or LamR295, which was nickel affinity-purified as described above for LamR37 (70°C). Each well was tested in triplicate, and binding affinity was determined by aspirating the next day. 200 µl of unsupplemented medium and 200 µl of luciferin substrate (SteadyGlo luciferase assay, Promega) were added. The cells were shaken vigorously for 15 min. The relative luciferase units, which correspond to infectivity of Sindbis virus vector, of each sample were read using a luminometer (Glomax 20/20, Promega). Experiments were performed in triplicate. A two-tailed Student’s t test was performed (p < 0.05) to determine statistical significance.

RESULTS AND DISCUSSION

LamR Expression, Purification, Crystallization, and Structural Determination—To better understand the function of LamR and its interaction with binding partners, we sought to crystallize the human LamR precursor protein. To this end, several different-length constructs were overexpressed in E. coli, including residues 1–295 (full-length), residues 1–220, and residues 1–195. Of these, only the 220-residue version of

| TABLE 1 LamR220 data collection and refinement statistics (molecular replacement) |
|---------------------------------------------------------------|
| **Data collection**                                           |
| Space group                                                   | P4_2_2 |
| Cell dimensions                                              |
| a, b, c (Å)                                                   | 75.69, 75.69, 99.01 |
| α, β, γ (°)                                                   | 90.00, 90.00, 90.00 |
| Resolution (Å)                                               | 50.0 (2.15) |
| Rmerge or Rmerge (%)                                          | 14.4 (47.7) |
| I/σI                                                         | 5.0 (4.0) |
| Completeness (%)                                              | 100 (100) |
| Redundancy (%)                                                | 94.9 (9.7) |
| **Refinement**                                               |
| Resolution (Å)                                               | 50.0 (2.15) |
| No. of reflections                                           | 14762 |
| Rwork/Rfree (%)                                               | 18.5/22.4 |
| No. of atoms                                                 | 1712 |
| Protein                                                      | 1563 |
| Ligand/ion                                                   | 0 |
| Water                                                        | 149 |
| Average B-factor                                             | 21.8 |
| r.m.s. deviations                                            | 0.009 |
| Bond lengths (Å)                                             | 1.22 |

*Values in parentheses are for highest resolution shell (2.25–2.15 Å).

* r.m.s., root mean square.
LamR (abbreviated LamR220) could be purified in amounts suitable for crystallization trials. LamR220 binds laminin-1 in vitro (supplementary Fig. 1a) and inhibits Sindbis virus vector infection of baby hamster kidney (BHK) cells (supplementary Fig. 1b). These data demonstrate that the first 220 residues of LamR are sufficient for interacting with key binding partners.

Crystals of LamR220 were obtained at pH 6.0 and belong to tetragonal space group P4_2_2_ with one molecule in the asymmetric unit. The crystal structure of LamR220 was determined by molecular replacement, using the crystal structure of the 30 S ribosomal protein S2p from A. fulgidus (PDB code 1VI6) as a search model (16). Data collection and refinement statistics at 2.15 Å resolution are given in Table 1.

Overall Structure of Human LamR—Consistent with a sequence identity of 32% between LamR and A. fulgidus S2p (residues 15–183) (Fig. 1), the two proteins share a similar overall architecture, classified (Structural Classification of Proteins (SCOP)) as an α/β protein with a flavodoxin-like fold (Fig. 2a). A central β sheet composed of five parallel β strands (β3–β7) is flanked by three α helices on one side (αB, αC, and αE) and a single α helix (αD) on the other side. An N-terminal α helix (αA) and two anti-parallel β strands (β1–β2) pack against the α/β core of the protein. Residues 1–8 and 206–220 of LamR220 are disordered in the structure.

Structural Comparison of Human LamR and S2 Protein from Other Species—Superimposition of the structures of LamR220 and A. fulgidus S2p yields a root-mean-square deviation in Cα positions of just 0.9 Å (174 atoms) and reveals two areas in which the structures are divergent (Fig. 2b): a segment between β4 and β5 (residues 111–118 in LamR) and a segment after the last α helix (αE) (residues 188–196 in LamR), in which LamR contains a five-residue insertion relative to A. fulgidus S2p. The segment between β4 and β5 contains an equal number of residues in the two proteins. In A. fulgidus S2p, the segment is stabilized in a folded-back conformation via a salt bridge between Arg-113 in this segment (Arg-117 in LamR) and Asp-93 (β4), the latter of which is not conserved in LamR (Thr-97). In the LamR220 structure, this segment instead projects away from the domain and packs against the same segment in a symmetry-related (two-fold) molecule (Fig. 2c). In this crystallographic dimer, Ala-114 packs into a tight pocket in the symmetry-related molecule formed by the β4–β5 segment and the end of αD, and Phe-116 is in van der Waals contact with Tyr-139 (αD) (Fig. 2c). Although Phe-116 is generally conserved from Saccharomyces cerevisiae through vertebrate species, Ala-114 is conserved only in vertebrates. The total surface area buried in this interface is a modest 832 Å², and LamR220 runs as a monomer in solution, but in the context of a membrane attachment and a possible covalent dimerization linkage (9), this crystallographic dimer could be functionally significant.

Functional Domains of LamR—The structural differences noted between LamR220 and A. fulgidus S2p (Fig. 2b) could be important for ribosomal protein function or for the acquired function as the receptor for laminin. Analysis of the 3.0 Å res-
olution structure of the 30 S ribosomal subunit from *Thermus thermophilus* (PDB code 1J5E) (21) indicates that the two major structural deviations between human LamR220 and *A. fulgidis* S2p (between β4 and β5 and after αE) would not appear to affect ribosomal function, since no RNA or protein contacts are present in these regions. This suggests that the structural differences in human LamR versus *A. fulgidis* S2p are important for laminin binding.

Previously, peptide segments of LamR utilized in binding assays implicated a segment, residues 161–180, known as peptide G, as a binding epitope for laminin (22). In the LamR220 crystal structure, this stretch of residues comprises the linker between β7 and most of αE. The only portion of this sequence that is solvent-accessible are residues 165–169 in the β7–αE linker. It is conceivable that the C-terminal tail of LamR220 undergoes a conformational change, exposing residues within this segment. Alternatively, the use of peptide segments of LamR to map the laminin binding site may need to be re-evaluated. In addition, a short putative transmembrane segment, residues 86–102, was suggested (22). This segment, encompassing β4 and most of αC, is an integral part of the protein fold and is unlikely to serve as a transmembrane helix.

**Role of LamR in Disease**—LamR has been implicated in a variety of diseases, including neoplastic disorders, Creutzfeldt-Jacob disease, urinary tract infections, encephalitis, and others. In cancer, the specific inhibition of LamR function at the cell surface of tumor cells, either by binding of EGCG, which most likely competes with endogenous laminin, or by infection with Sindbis virus vector, which is internalized by receptor-mediated endocytosis, has been associated with anti-tumor effects (23–26). Both EGCG and Sindbis virus vectors, through two different mechanisms, reduce the ability of LamR at the surface to interact with laminin. The structural characterization of LamR will contribute to an understanding of how LamR interacts with its binding partners and aid in the development of therapeutics that can block and/or mimic LamR interactions in the setting of cancer, neurological disorders, and viral infection.

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