Cell Tissue Res (1996) 286:487–491

Cell & Tissue Research

Localization of histidyl-tRNA synthetase (Jo-1) in human laryngeal epithelial carcinoma cell line (HEp-2 cells)

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Abstract. The present study was designed to determine the subcellular localization of histidyl-tRNA synthetase (Jo-1) in human laryngeal epithelial carcinoma cell line (HEp-2 cells). Indirect immunofluorescence using commercial HEp-2 cells with human serum and human-affinity-purified anti-Jo-1 antibodies was performed using confocal microscopy. Anti-histidyl-tRNA-synthetase-positive sera showed distinct nuclear and cytoplasmic granular staining in HEp-2 cells. Affinity purified anti-Jo-1 produced an identical pattern to the whole serum, whereas the serum fraction that did not bind to the affinity column was negative by immunofluorescence on HEp-2 cells. Two commercial human anti-Jo-1-positive control sera and seven anti-Jo-1-positive sera from patients with myositis reproduced the nuclear and cytoplasmic granular pattern. We conclude that Jo-1 is present in cytoplasm and in intact nuclei from HEp-2 cells. The presence of tRNA synthetases in intact nuclei suggests that they have an unsuspected function in the nucleus.

Key words: Autoantibodies – Human anti-Jo-1 antibodies – Myositis – Confocal microscopy – Human

Introduction

Myositis is an autoimmune disease characterized by infiltrates of inflammatory cells in the skeletal muscle and the presence of specific antibodies (Medsger and Oddis 1994). Anti-histidyl-tRNA synthetase (anti-Jo-1) antibodies are found in patients with myositis, especially those with lung involvement (Medsger and Oddis 1994). Anti-Jo-1 antibody recognizes histidyl-tRNA synthetase (Jo-1), a dimer of 50 kDa subunits found in the cytoplasm of human cells (Targoff 1992; Medsger and Oddis 1994). In the first characterization of human anti-Jo-1, Nishikai and Reichlin (1980) found Jo-1 antigen in the nucleus using chromatographically purified antigen from nuclear and whole calf thymus extracts. They then obtained affinity-purified Jo-1 using a Sepharose column coupled to the IgG fraction of the anti-Jo-positive serum (containing anti-Jo-1 and anti-Jo-2). By double immunodiffusion, the purified Jo-1 showed a line of identity with whole and nuclear calf extracts in the reaction with anti-Jo. A strong nuclear speckled pattern was obtained using mouse spleen cells as substrate and a concentration of anti-Jo IgG greater than 0.4 mg/ml. However, when using mouse liver as substrate, no reaction was seen (Nishikai and Reichlin 1980). Shi et al. (1991) found Jo-1 in the cytoplasm using indirect immunofluorescence on cultured human myoblasts. Whole anti-Jo-1 serum demonstrated both granular and cytoplasmic staining, but only the cytoplasmic staining was inhibited by preabsorbing the sera with Jo-1, suggesting that Jo-1 is located only in the cytoplasm (Shi et al. 1991). It has been suggested that the nuclear localization seen with human cells is due to contamination of the nuclear preparations with cytoplasmic proteins. Anti-Jo-1 produced only the cytoplasmic staining on rodent cells (Dan et al. 1986). Other aminoacyl-tRNA synthetases have been localized to nuclei as well as to the cytoplasm (Filonenko and Favorova 1991; Barbarese et al. 1995), suggesting a nuclear function possibly related to transcription, processing or transport for these enzymes (Uyttenbroek et al. 1993).

Since the subcellular localization of Jo-1 is controversial and generally Jo-1 is thought to be solely cytoplasmic (Nishikai and Reichlin 1980; Dan et al. 1986; Shi et al. 1991; Uyttenbroek et al. 1993), blots on HeLa chromosomes or nuclei are not routinely used to detect the anti-Jo-1 antibody (Nishikai and Reichlin 1980; Dan et al. 1986; Targoff 1992; Medsger and Oddis 1994). We recently found that Western blot analysis of human HeLa nuclear extracts with an anti-Jo-1 positive control (Center for Disease Control in Atlanta, Ga., USA) revealed a band at 50 kDa (Vázquez-Abad and Rothfield

This work has been supported by grants from the National Institutes of Health K08-AR01954-01 to D.V.A., AR37986 and AR20621 to N.F.R., and NS15190 to J.H.C.

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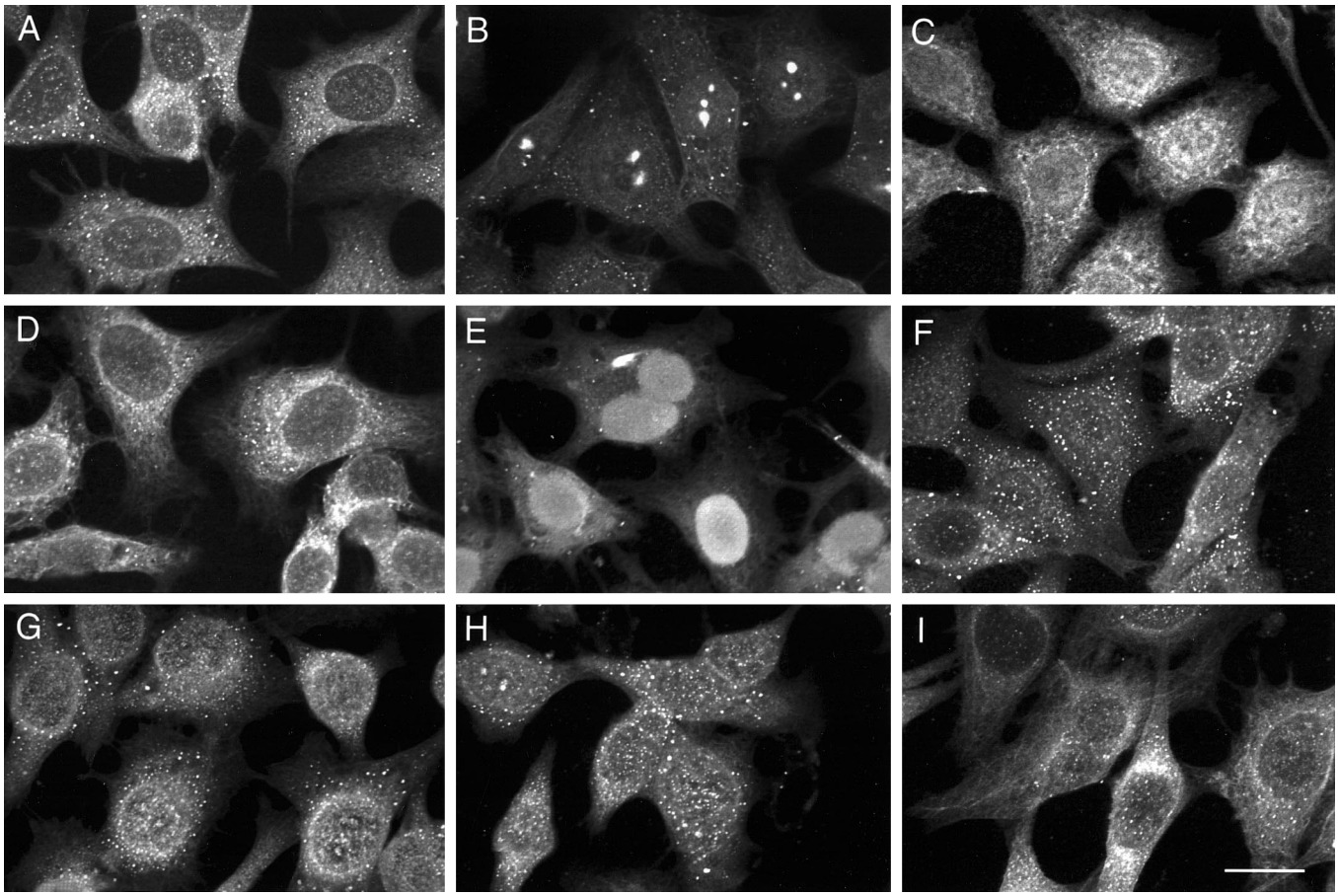

Fig. 1A–I. Immunofluorescent labeling of human laryngeal epithelial carcinoma cell line (HEp-2 cells) with various sera from patients with myositis. HEp-2 cells were labeled with sera from seven different patients with myositis (**A–G**). In addition, commercial anti-histidyl-tRNA-synthetase (anti-Jo-1) antisera from Immunovision (**H**) and from alpha antigens (**I**) were used. *Scale bar*: 20 μm

1996). This observation led us to carry out further studies to clarify the localization of Jo-1 by indirect immunofluorescence on human laryngeal epithelial carcinoma cell line (HEp-2 cells) using confocal microscopy. The confocal microscope measures the distribution of antigen within a narrow optical section through the cell without interference from cellular components above or below the optical section. The thickness of the optical section (approximately 1 μm) is much less than the diameter of the nucleus (approximately 10 μm). Therefore, the fluorescent signal within the cross-sectional profile of the nucleus represents the distribution of antigen within the nucleus, and the signal outside the profile of the nucleus represents the distribution of antigen within the cytoplasm. Using confocal immunofluorescence microscopy, Jo-1 autoantigen was detected in both cytoplasm and nucleus of HEp-2 cells.

Materials and methods

Sera

Sera from seven anti-Jo-1-positive myositis patients were studied (Vázquez-Abad and Rothfield 1996). These patients were seen in the Arthritis Clinics at the University of Connecticut School of Medicine, Farmington, Conn., USA. All human studies had been approved by the appropriate ethics committee and have been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Informed consent was given, and the signed consent forms were obtained before serum from each patient was collected and kept at −70° C. A monospecific anti-Jo-1-positive serum from the Center for Disease Control in Atlanta, Ga., USA was used as a positive control for all experiments. In addition, we tested anti-Jo-1-positive sera from two commercial sources (Immunovision, Springdale, Ark., USA; Alpha Antigens, Columbia, Mo., USA) as well as affinity-purified anti-Jo-1 antibody. A pool of normal human serum devoid of autoantibodies (NHS) by Western blot analysis on HeLa nuclei was used as a negative control (Vázquez-Abad and Rothfield 1996).

Affinity purification of anti-Jo-1

Five hundred units of affinity-purified Jo-1 antigen (Immunovision, Springdale, Ark., USA) were coupled to CH-activated Sepharose 4-B (Pharmacia, Uppsala, Sweden) as previously described (Vázquez-Abad et al. 1993). Anti-Jo-1 serum (CDC, Atlanta, Ga., USA) was exhaustively absorbed on the Jo-1-coupled Sepharose 4B gel, and pH neutralized immediately after acid elution. The non-bound fraction was used as a negative control (Vázquez-Abad et al. 1993).

Smaller amounts of anti-Jo-1 were also affinity purified from HeLa blots. After transfer to nitrocellulose paper, the HeLa nucle-

ar blots were stained with Ponceau-S red, and the 50-kDa band identified by the CDC anti-Jo-1 was cut in <3-mm strips (Vázquez-Abad and Rothfield 1996). After a 30-min incubation in phosphate-buffered saline (PBS), pH 7.4, wiht 4% bovine serum albumin (BSA, Sigma, St. Louis, Mo., USA) the strips were incubated overnight at 4° C with the CDC anti-Jo-1 sera at 1:500 in the same buffer. After thorough washes with PBS, the elution was performed by adding 800 µl of 0.2 M HCl-glycine buffer, pH 2.2, with 0.1% gelatin for 5 min at 0° C. The pH was neutralized with 250 µl of 1 M TRIS buffer (pH 8) and the eluate was dialyzed against PBS and used for indirect immunofluorescence.

Indirect immunofluorescence on HEp-2 cells

Indirect immunofluorescence on HEp-2 cells (Incstar Co, Stillwater, Minn., USA) was performed as previously described (Tramposch et al. 1984). Briefly, cells were thawed for 15 min at room temperature and then incubated with sera diluted at 1:20 in PBS for 30 min. After thorough washes with PBS, the slides were incubated with fluorescein-labeled anti-human antibodies (Incstar) for 30 min, and, after thorough washes with PBS, the slides were covered and kept at 4° C until they were studied by fluorescent microscopy.

Confocal microscopy

Confocal images of representative fields of HEp-2 cells were collected. The focal plane was set to pass through the center of the nucleus so that Jo-1 antigen could be detected in both nucleus and cytoplasm within the same cell. The imaging system used was an MRC600 confocal laser scanning system with dual photomultipliers attached to a Zeiss Axioskop upright microscope equipped with several high numerical aperture objectives. A Silicon Graphics Iris 340VGX supercomputer was used for image processing and three-dimensional visualization (Ainger et al. 1993).

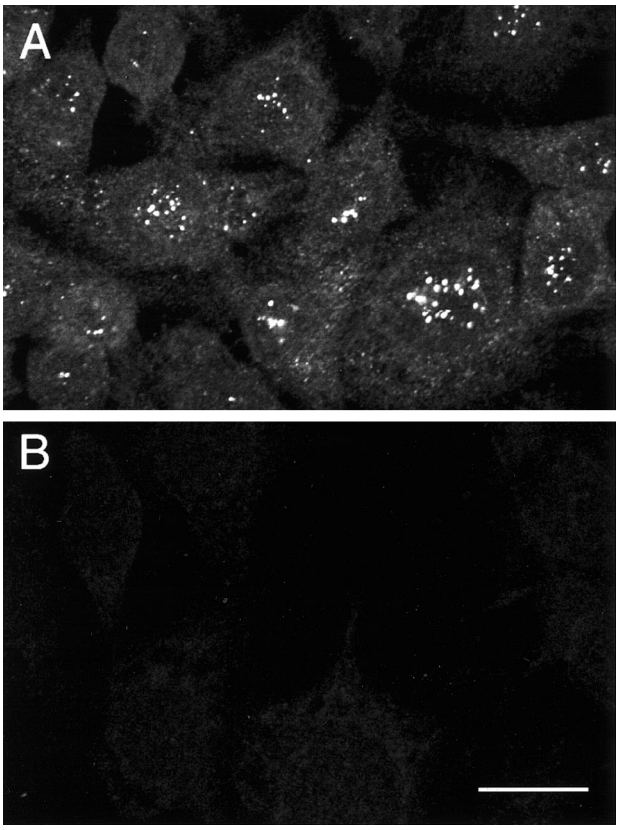

Fig. 2A, B. Immunofluorescent labeling of HEp-2 cells with affinity-purified anti-Jo-1 antibody. Anti-Jo-1 antibody was affinity purified as described in 'Materials and methods'. The affinity-purified fraction (**A**) and the non-bound fraction (**B**) were used for immunofluorescent labeling of HEp-2 cells. *Scale bar*: 20 µm

Results

The CDC anti-Jo-1 serum showed cytoplasmic and nuclear labeling by indirect immunofluorescence on HEp-2 cells (results not shown). Serum samples from seven anti-Jo-1-positive patients also produced granular nuclear and cytoplasmic labeling similar to the pattern produced by the CDC anti-Jo-1 serum (Fig. 1), although the intensity of labeling varied among the different sera. Some of the seven patients' sera appeared to stain other cellular components besides the granular anti-Jo-1 labeling. For example, Fig. 1C shows fibrillar intracytoplasmic labeling, and Fig. 1E shows diffuse labeling in the nucleus. The confocal images shown in Fig. 1 are eight-bit digital images that were collected with the gain and black level settings adjusted to maximize the dynamic range within each image. This means that the most intensely labeled component in the cell appears to an intensity value of 255, and other labelled components have proportinately lower intensities. Since some sera label particular cellular components more intensely than Jo-1 in these images, the granular Jo-1 labeling appears less intense than in images where Jo-1 is the most intensely labeled component in the cell. Two commerical anti-Jo-1 sera also produced patterns similar to that of the CDC control serum (Fig. 1H, I).

Preabsorption studies showed that the affinity-purified anti-Jo-1 (CDC) stained coarser nuclear than cytoplasmic granules (Fig. 2A). The molecularly purified anti-Jo-1 from the HeLa blots produced exactly the same staining on HEp-2 cells (not shown). The non-bound fraction from the Jo-1 affinity column produced no fluorescence in the nucleus or cytoplasm (Fig. 2B).

The detailed three-dimensional morphologies of cytoplasmic and nuclear Jo-1 were analyzed using isosurface visualization techniques (Morgan et al. 1992). Cells were stained with affinity-purified anti-Jo-1 antibody and optically sectioned with the confocal microscope. The series of optical sections was reconstructed into a three-dimensional matrix and subvolumes containing Jo-1 antigen were delineated using a marching cubes algorithm. Multiple isosurfaces were rendered using Gourand shading and lighting. A representative cell is shown in Fig. 3. The upper surface of the cell has been computationally clipped away in some regions to reveal the distribution of Jo-1 within the cell. In the cytoplasm, the synthetase is distributed in a dense reticulum in which are embedded numerous small granules. The granular are generally less than 1 µM in diameter and the concentration of synthetase is higher in the granules than in the surrounding reticulum. Similar aminoacyl-tRNA-synthetase-containing granules have been reported in other cell

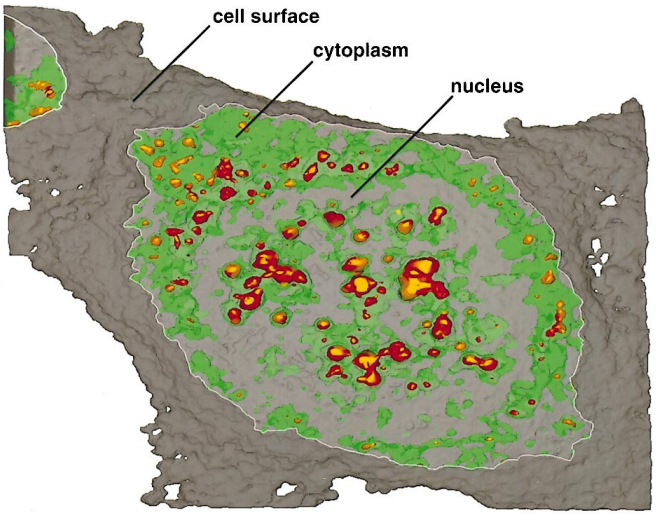

Fig. 3. Three-dimensional visualization of the subcellular distribution of histidyl-tRNA synthetase (Jo-1) in HEp-2 cells. HEp-2 cells were labeled with affinity-purified anti-Jo-1 antibody. The cell was optically sectioned at 0.5-μm intervals with confocal microscope. The series of optical sections was reconstructed into a three-dimensional mesh of voxels, and various isosurfaces were calculated using a marching cubes algorithm. An isosurface at an intensity of 3, which corresponds to the diffuse autofluorescence within the cell (rendered in *opaque gray*), delineates the cell surface. A clipping plane is used to cut away portions of the cell surface exposing the interior of the cytoplasm and nucleus. A second isosurface at an intensity of 30 (rendered in *transparent green*) delineates the reticular distribution of antigen in the cytoplasm and nucleus of the cell. A third isosurface at an intensity of 50 (rendered in *opaque red with yellow highlights*) delineates granular cytoplasmic and globular nucleoplasmic concentrations of synthetase embedded in the reticulum. The positions of the cell surface, cytoplasm and nucleus are indicated. No scale bar is included because of perspective effects within the three-dimensional image. The nucleus is approximately 20 μm in diameter

types and have been shown to contain several different components of the protein translational machinery (Barbarese et al. 1995). The distribution of Jo-1 in the nucleus is somewhat different to that in the cytoplasm. In the nucleus, the synthetase is distributed in a sparser reticulum than in the cytoplasm and several large globular concentrations of synthetase are embedded in the reticulum. The nature of the synthetase globules, which are generally greater than 1 μm in diameter, is not known. They do not appear to correspond to nucleoli since there are 10–20 globules in the nucleus, as shown in Fig. 3, whereas these cells contain 2–7 nucleoli. The different morphological appearance of Jo-1 in the cytoplasm and nucleus suggests that the enzyme is organized differently and may perform different functions in the two subcellular compartments.

Discussion

In the present study we found that the CDC control anti-Jo-1 serum showed a granular labeling pattern in both nuclei and cytoplasm of HEp-2 cells by indirect immunofluorescence. The staining of cytoplasmic and nuclear granules suggests that the Jo-1 autoantigen is present in both cellular compartments. However, the different appearances of the granules in the two compartments suggest that Jo-1 is organized differently in the nucleus than it is in the cytoplasm. The different patterns produced by the patients' serum samples may be explained by the fact that whole sera from these patients contain other autoantibodies that bind different components of the human HEp-2 cells. Immunoblots on HeLa cells showed that these sera had other bands along with the characteristic 50-kDa Jo-1 band (Vázquez-Abad and Rothfield 1996).

The confocal immunofluorescence data presented here indicate that the Jo-1 antigen is present in granular structures in both the nucleus and the cytoplasm of fixed HEp-2 cells. Previous work has reported diffuse cytoplasmic staining because conventional microscopy cannot resolve fine structures in detail. Subcellular localization studies using immunofluorescence on fixed cells can, under some conditions of fixation, be artifactual because of redistribution and/or differential extraction of soluble proteins (Melan and Sluder 1992). It is unlikely that the localization of Jo-1 antigen reported here is artifactual because the distribution pattern is similar to the distribution of arginyl-tRNA synthetase in oligodendrocytes under different fixation conditions (Barbarese et al. 1995). In the oligodendrocyte study, the granular structures in the cytoplasm were shown to contain, besides tRNA synthetase, elongation factor 1a, ribosomes, and mRNA, and were postulated to contain all the components necessary for protein synthesis. It is likely that the cytoplasmic Jo-1-containing granules reported here represent similar protein synthetic granules in HEp-2 cells. The nucleus of the HEp-2 cells also had Jo-1 containing granules that appeared coarser than the cytoplasmic granules. In the oligodendrocyte study, arginyl-tRNA synthetase was also localized in the oligodendrocyte nucleus (Barbarese et al. 1995). Taken together, these results suggest that the nucleus may contain many, if not all, aminoacyl-tRNA synthetases. The function of aminoacyl-tRNA synthetase in the nucleus is not yet known. Aminoacyl-tRNA synthetases may have a function related to tRNA transcription, processing, or transport in the nucleus of the cells. Further studies to answer this question should include the identification of other components within the Jo-1 nuclear globules to elucidate its possible functions.

Acknowledgements. We appreciate the helpful discussions and technical suggestions of Murray Deutscher, and the excellent technical help of Lesley S. Daniels and Frank Morgan.

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