Distribution of Gap Junction Protein Connexin 37 in Smooth Muscle Cells of the Rat Trachea and Pulmonary Artery*

Kyoko NAKAMURA, Tetsuichiro INAI, Keiichiro NAKAMURA and Yosaburo SHIBATA

Department of Anatomy, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Received October 26, 1998

Summary. Connexin 37, one of the gap junction protein families, has been detected by Northern blotting in various organs and tissues, and found to be especially abundant in the lung. However, detailed information on the precise types of cells which express connexin 37 has not been previously published. We therefore prepared site-specific connexin 37 antibodies and examined the distribution of connexin 37 immunohistochemically. Connexin 37 was detected in endothelial cells, in the tunica media of both the pulmonary artery and the aorta, and in the smooth muscle layer of the trachea and bronchioles. In the tracheal smooth muscle layer, connexin 37 overlapped with desmin-positive areas, but was clearly segregated from vimentin- and von Willebrand factor-positive areas. These results suggest that connexin 37 is expressed in smooth muscle cells in the trachea, but not in fibroblastic cells or endothelial cells. Connexin 37 was partially colocalized with connexin 43 in tracheal smooth muscle cells, and showed a gradual increase in expression during postnatal development. To our knowledge, this is the first report to be published regarding the expression of connexin 37 in smooth muscle cells.

Gap junctions are clusters of transmembrane protein channels through which small molecules of less than 1,000 Da, such as cAMP, can pass from the cytoplasm of one cell to that of a neighboring cell (for reviews see KUMAR and GILULA, 1992; BEYER, 1993; HALL and GOURDIE, 1995; WOLBURG and ROHLMANN, 1995; YAMASAKI and NAUS, 1996). Intercellular communication through gap junctions plays an important role in the maintenance of the normal function of organs, embryonic differentiation, and growth control in multicellular organisms (LOEWENSTEIN, 1988; GUTHRIE and GILULA, 1989). Each gap junction channel is formed by an interconnection of hemichannels consisting of hexamer subunit proteins called connexin (Cx). At least twelve connexin proteins with homologous amino acid sequences have been cloned and characterized in mammals. Different connexin proteins are expressed in different types of cells, tissues, and species (for reviews see BRUZONE et al., 1996; KUMAR and GILULA, 1996). Each connexin has its own channel properties (ELFGANG et al., 1995). Such diversity in mammalian cells suggests that each connexin is functionally specialized.

Cx37, a member of the connexin family, was initially cloned from the mouse brain, and later detected abundantly by northern blot analysis in the mouse lung (WILLECKE et al., 1991) and the rat lung (HAEFLINGER et al., 1992). However, the precise localization of Cx37 has not been fully determined, partially due to the complicated structure of lung tissue. It was reported that Cx37 is also expressed in endothelial cells of the rat aorta and coronary artery (YE et al., 1997). In order to examine the precise localization of Cx37, we prepared site-specific antibodies against Cx37 and performed immunohistochemical examinations using various tissues.

In this study, we found—for the first time—that Cx37 is expressed in the smooth muscle cells of the rat trachea and large arteries. We also examined the expression patterns of Cx37 during development.

MATERIALS AND METHODS

Preparation of polyclonal and monoclonal anti-Cx37 antibodies

Site-specific polyclonal and monoclonal antibodies for Cx37 were prepared against synthetic oligopeptide sequences corresponding to the carboxy-terminal amino acid residues (304–318: RPPPVNVTAFQGQK).

*This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.
of mouse Cx37 (Willecke et al., 1991). The synthetic peptide was conjugated to keyhole limpet haemocyanin (KLH; Calbiochem, La Jolla, CA) through bifunctional crosslinker GMBS (N-c-maleimido-butyryloxysuccinimide ester; Calbiochem). Japanese white rabbits were immunized by means of subcutaneous injections at multiple sites along the back a total of three times at 2 or 3-week intervals with 1 mg of peptide which had been mixed with the same volume of Freund's complete adjuvant (Difco Laboratories, Detroit, MI). Antisera were collected 1-2 weeks after the final injection. Specific immunoglobulins from the antisera were affinity-purified using the antigen peptides coupled to Affigel-10 (Bio-Rad Laboratories, Richmond, CA) according to the supplier's protocol.

Balb/c mice were also immunized with the same antigen, but by intraperitoneal injections in the manner described above. Splenocytes were collected from the immunized mice and fused with mouse myeloma cells, P3U1, using polyethylene glycol 4000. After HAT (hypoxanthine, aminopterin and thymidine) selection, the culture media from the wells containing macroscopically visible colonies were screened by indirect immunofluorescence using frozen sections of the rat lung. Hybridomas producing antibodies, which revealed punctuate spots between the endothelial cells of the arteries, were cloned three times by limiting dilutions. Monoclonal anti-Cx37 antibody was collected from the ascites of the mouse after peritoneal injection with the hybridoma cells.

**Immunoblot analysis**

Crude gap-junction membranes were prepared according to Hertzberg's alkaline extraction method (1984), with some modifications. Proteins separated by electrophoresis were transferred electrophoretically to nitrocellulose sheets at 100 V for 1 h (Towbin, et al., 1979). After blocking with 5% (w/v) skim milk (Difco) in PBS containing 0.1% (v/v) Tween-20 (T-PBS/milk) for 1 h, the blots were incubated with the primary antibodies, and diluted to 0.2 μg/ml with T-PBS/milk at room temperature for 1 h. They were then washed six times for 5 min in T-PBS, and incubated for 1 h at room temperature with horse-radish peroxidase (HRP)-labeled goat, anti-rabbit or anti-mouse IgG (Bio-Rad Laboratories) diluted 1:2,000 with T-PBS/milk. The localization of peroxidase was detected using enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham), and exposed to Hyperfilm (Amersham).

**Northern blot analysis**

Total RNAs were isolated from the adult rat trachea and lung using a single-step thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). The concentration of RNA was determined by measuring absorption at 260 nm. Twenty micrograms of RNA per lane were run on a 1% agarose/formaldehyde gel. Some gels were stained with 20×SSC containing 0.5 μg/ml ethidium bromide. Other gels were capillary-blotted in 20×SSC onto nylon membranes (Hybond N; Amersham), and fixed by baking at 80°C for 2 h in a vacuum oven. Membranes were pretreated with 50% (v/v) formamide, 5×SSPE, 0.5% (w/v) SDS, and 5×Denhardt's solution at 37°C for 2 h. The blots were then hybridized overnight at 42°C in the same buffer containing α-32P dCTP (Du pont/NEN Research Products, Boston, MA) labeled probes. The following probes were used for hybridization: 1) Cx43 cDNA fragment (Eco RI, 1400bp) (Beyer and Paul, 1987); and 2) Mouse Cx37 DNA probe (739-1063) which was generated by the RT-PCR method, its sequence being confirmed by dieoxy sequencing. Stringent washings were performed repeatedly—twice for 10 min at 70°C with 2×SSC and 0.1% (w/v) SDS, followed by another two times for 20 min at 65°C with 1×SSC and 0.1% SDS, and then a further two times for 20 min at 65°C with 0.1×SSC and 0.1% SDS. Autoradiographs were obtained by exposing the hybridized blot to an imaging plate and analysed with BAS-2000 (Fujix, Tokyo).

**Antibodies**

The following monoclonal and polyclonal antibodies (mAb and pAb, respectively) were used: mouse anti-Cx37 mAb, rabbit anti-Cx37 pAb, mouse anti-Cx43 mAb (Zymed Laboratories, San Francisco, CA), mouse anti-desmin mAb (DAKO, Denmark), mouse anti-vimentin mAb (Sigma Chemical Co., St. Louis, MO), and rabbit anti-von Willebrand factor pAb (Dakopatts, Denmark).

**Immunofluorescence microscopy**

For indirect immunohistochemistry, 19-20 days postcoitus (E19-20), 1-week-old, 3-week-old, and adult rat tracheas were frozen in a liquid nitrogen-cooled OCT compound (Tissue Tek; Miles, Elkhart, IN). The lung, pulmonary artery, aorta, stomach, small intestine, nonpregnant uterus, epididymis, ductus deferens, urethra and urinary bladder of adult rats also underwent preparation. Cryosections, 5 μm thick, were mounted on glass slides, immersed in absolute acetone at −20°C for 10 min, rinsed for 5 min three times in PBS, and then incubated with 1% (w/v) BSA (bovine serum albumin) in PBS for 30 min at room temperature to reduce non-specific binding. The sections were then incubated for 1 h at room tempera-
Fig. 1. Immunoblot analysis of rabbit polyclonal and mouse monoclonal antibodies raised against amino acid residues 304–318 of mouse Cx37 and mouse anti-Cx43 monoclonal antibody. Crude membrane proteins from the rat lung (lane a, b) and heart (lane c) were fractionated by SDS-PAGE and transferred to nitrocellulose sheets. These were then probed with rabbit anti-Cx37 pAb (a), with mouse anti-Cx37 mAb (b), and with mouse anti-Cx43 mAb (c). Immunoreactive bands were visualized by ECL system (Amersham) after labeling with HRP-conjugated secondary antibodies. Mobility of the molecular mass standard is indicated in kilodaltons (kDa). A single protein band with a relative molecular mass of 37 kDa can be detected in lanes a and b. A molecular mass of 41–45 kDa can be observed in lane c.

Fig. 2. Northern blot analysis of the adult rat trachea and lung. Total RNAs (20 μg) were fractionated by electrophoresis in a 1% agarose-formaldehyde gel, transferred to a nylon membrane, and then hybridized with α-32P dCTP-labeled probes against Cx37 (a), and Cx43 (b). Some gels were stained with ethidium bromide (c). Lane 1: rat trachea, lane 2: rat lung. Cx37 probe revealed 1.5 kb transcripts and Cx43 probe revealed 3.6 kb transcripts in both the trachea and the lung.
from adult rats were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at room temperature for 7 h. Specimens were postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer and stained en bloc with 4% uranyl acetate at room temperature before being embedded in Epon 812. Ultrathin sections were doubly stained with uranyl acetate and lead citrate, and observed through a JEOL 2000 EX electron microscope (Jeol, Tokyo, Japan) at an accelerating voltage of 100 kV.

RESULTS

Immunoblot analysis

Rabbit and mouse antibodies raised against residues 304-318 of mouse Cx37 were characterized by immunoblot analysis using membrane fractions of the rat lung. Immunoblotting with rabbit and mouse anti-Cx37 antibodies revealed a single protein band with a relative molecular mass of 37 kDa (Fig. 1 lane a,b). A band whose molecular mass was 41-45 kDa was detected by mouse anti-Cx43 mAb using membrane fractions prepared from the rat heart (Fig. 1 lane c). It is known that Cx43 has phosphorylation sites; therefore, the higher molecular mass detected by the mouse anti-Cx43 mAb may have been phosphorylated forms of Cx43 protein. These results indicate that the antibodies used in this study specifically react with either Cx37 or Cx43 protein. Only faint bands of Cx37 and Cx43 proteins were detected within membrane fractions prepared from the rat trachea, probably due to the small amount of gap junction membranes within the loaded proteins (data not shown).

Northern blot analysis

The total RNAs isolated from the trachea and lung of the rats were fractionated and hybridized with 32P-labeled cDNA probes of Cx37 and Cx43. A single transcript of 3.6 kilobases (kb) was detected by the Cx43 probe and a 1.5 kb transcript was found by the Cx37 probe in both the trachea and the lung (Fig. 2). The signals of both Cx37 and Cx43 were stronger in the lung than in the trachea. There results demonstrate that mRNAs encoding Cx37 and Cx43 are expressed in the trachea.

Immunofluorescence microscopy

In order to clarify the precise localization of Cx37 in the lung, indirect immunofluorescence was performed. Non-specific staining was consistently observed in elastic fibers (Figs. 3-5, 10). Cx37 spots were detected in the endothelial cells of small arteries (Fig. 3a, b) however, they were not detected in alveolar epithelium. Cx37 spots and Cx43 spots were both detected in the smooth muscle layer of the bronchiole (Fig. 3d, f); they were not colocalized with each other (Fig. 3e) however. In small arteries, Cx43 spots could not be detected in the media or endothelial cells (Fig. 3c), though non-specific staining was recognized in the elastic fibers of the arterial media and bronchiole.

In the pulmonary artery, Cx43 and Cx37 spots were both detected in endothelial cells and in the smooth muscle layer (Fig. 4a, c). They were almost always colocalized in the former, but were only partially colocalized in the latter (Fig. 4b). In the pulmonary vein, Cx37 spots were not detected in either the endothelial cells or the smooth muscle layer (data not shown).

Epithelium of the trachea and elastic fibers of the lamina propria revealed non-specific staining using only TRITC-labeled anti-rabbit IgG (Fig. 5b) or FITC-labeled anti-mouse IgG (Fig. 5c). In this study, observations were confined to the smooth muscle layer only. Cx37 and Cx43 both existed within the smooth muscle layer of the trachea (Fig. 5d, e). The partial colocalization of Cx37 and Cx43 is shown in Figure. 6. Cx37 spots were observed at the edge of the areas stained with anti-desmin mAb (Fig. 7), but were clearly segregated from those areas stained with both anti-von Willebrand factor (data not shown) and anti-vimentin mAb (Fig. 8).

Electron microscopy of tracheal smooth muscle

In order to confirm that gap junctions are present between smooth muscle cells of the trachea, electron microscopic observations were undertaken. Gap junctions were observed between smooth muscle cells characterized by thin filaments, caveolae, and a basement membrane (Fig. 9). Tracheal smooth muscle cells had many projections, and gap junctions were observed between the tips of these projections. A few fibroblastic cells that lacked any basement membrane were observed among the specimens. Gap junctions could not be detected between the fibroblastic cells themselves, or between the fibroblastic cells and the smooth muscle cells.

Distribution of Cx37 and Cx43 in various smooth muscles

To compare the Cx37 expression of smooth muscle cells in other tissues, we examined the immuno-
Fig. 3. Double immunolabeling of a frozen section of the adult rat lung stained with mouse anti-Cx43 mAb and rabbit anti-Cx37 pAb. A small artery of the lung is shown in a, b and c, and a bronchiole is shown in d, e and f. The localization of Cx37 is visualized in red with TRITC-labeled anti-rabbit IgG (a, b), while that of Cx43 is visualized in green with FITC-labeled anti-mouse IgG (c, f). Overlapping images of the two fluorescence probes are shown in b and e. Elastic fibers in the small artery and bronchiole reveal non-specific labeling. In the small artery, red spots for Cx37 are detected in endothelial cells just above the internal elastic lamina. Green spots for Cx43 are not detected in the small artery. A few red spots for Cx37 are expressed in the smooth muscle of the bronchiole, but they are not colocalized with the green spots for Cx43. Arrowheads in e indicate elastic fibers in the lamina propria just beneath the bronchial epithelium. L lumen, E epithelium, SM smooth muscle. Bars (c, f)=10 μm

Fig. 4. Double immunolabeling of a frozen section of the adult rat pulmonary artery stained with rabbit anti-Cx37 pAb (a), and mouse anti-Cx43 mAb (c). An overlapping image is shown in b. Yellow spots in b indicate colocalization of Cx43 and Cx37. Red spots for Cx37 in endothelial cells are almost colocalized with green spots for Cx43. Red spots for Cx37 in smooth muscle cells are partially colocalized with Cx43 spots. L lumen, E epithelial cell, SM smooth muscle of tunica media. Bar (c)=10 μm

reactivities for Cx37 and Cx43 in various tissues. Cx37 was detected in the trachea, pulmonary artery and aorta, but not in the stomach, small intestine, nonpregnant uterus, epididymis, ductus deferens, urethra or urinary bladder, whereas Cx43 was detected in the trachea, pulmonary artery, aorta, stomach, circular muscular layer of the small intestine and the epididymis. These results are summarized in Table 1.

Expression pattern of Cx37 and Cx43 in tracheal smooth muscle during development

In order to examine the perinatal development of gap junctions in the respiratory passage, E19-20, 1-week-
old and 3-week-old rat tracheas were double-stained with anti-Cx43 mAb and anti-Cx37 pAb. Cx37 spots were not detected in the E19-20 rats (Fig. 10b), and only a few Cx37 spots were detected in 1-week-old rats (Fig. 10f). However, the expression of Cx37 showed a considerable increase in the 3-week-old rats (Fig. 10j). In contrast to Cx37, Cx43 spots were consistently detected in E19-20, 1-week-old and 3-week-old rats (Fig. 10d, h, l). The developmental change of Cx37 was more dynamic than that of Cx43. In 1-week-old rats, some Cx37 spots were colocalized with Cx43 spots (Fig. 10g). In 3-week-old rats, some of the Cx37 spots were colocalized with Cx43 spots, but others remained segregated from the Cx43 spots (Fig. 10k).
Fig. 6. Double immunolabeling of a frozen section of the adult rat tracheal smooth muscle bands stained with rabbit anti-Cx37 pAb (a) and mouse anti-Cx43 mAb (c). An overlapping image is shown in b. Yellow spots indicate an overlapping image of two fluorescence probes (b). Some of the Cx37 spots are colocalized with Cx43 spots. Bar (c) = 20 μm

Fig. 7. Double immunolabeling of a frozen section of the adult rat tracheal smooth muscle bands stained with rabbit anti-Cx37 pAb (a) and mouse anti-desmin mAb (c). An overlapping image is shown in b. Red spots for Cx37 are detected at the edge of the green signals for desmin. Bar (c) = 20 μm

Fig. 8. Double immunolabeling of a frozen section of the adult rat tracheal smooth muscle bands stained with rabbit anti-Cx37 pAb (a) and mouse anti-vimentin mAb (c). An overlapping image is shown in b. Red spots for Cx37 are clearly segregated from the green signals for vimentin. Bar (c) = 20 μm
Table 1. Connexin expression in various smooth muscles

<table>
<thead>
<tr>
<th></th>
<th>Cx37</th>
<th>Cx43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Aorta</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Stomach</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>circular muscular</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>longitudinal muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>layer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Epididymis</td>
<td>-</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Ductus deferens</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urethra</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

-= negative, +: weak, ++: moderate, +++: strong

**DISCUSSION**

Previous immunohistochemical studies have demonstrated that Cx37 is expressed in the rat epidermis (GOLIGER and PAUL, 1994), in the endothelial cells of rat arterial tissue (YEH et al., 1997), and between oocytes and granulosa cells of the mouse (SIMON et al., 1997). The present study demonstrated for the first time that Cx37 is expressed in the smooth muscle cells of the trachea, pulmonary artery and aorta.

Cx37 was detected in the tracheal smooth muscle layer, which consisted of not only smooth muscle cells, but also endothelial cells and fibroblastic cells, among others. In order to clarify which types of cells express Cx37, double-immunostaining was carried out using anti-Cx37 Ab with either anti-desmin Ab, anti-vimentin Ab, or anti-von Willebrand factor Ab. The areas expressing desmin, vimentin, or von Willebrand factor may correspond to muscle cells, endothelial and fibroblastic cells, and endothelial cells, respectively. Cx37-positive spots were overlapped with desmin-positive areas but were clearly segregated from von Willebrand factor-positive areas and vimentin-positive areas. Accordingly, Cx37-positive spots would seem to correspond to the gap junctions of smooth muscle cells in the trachea, but not to those of endothelial cells or fibroblastic cells. In addition, the electron microscopic study revealed that gap junctions were easily detected between smooth muscle cells, but could not be detected between smooth muscle cells and fibroblastic cells. This is consistent with previous ultrastructural studies which frequently demonstrated gap junctions between the smooth muscle cells within the trachea (RICHARDSON and FERGUSON, 1979; DANIEL et al., 1986).

In the present study, Cx43 spots were detected...
Fig. 10. Double immunolabeling of a frozen section of E19–20, 1-week-old, and 3-week-old rat tracheas stained with the rabbit anti-Cx37 pAb (b, f, j), and the mouse anti-Cx43 mAb (d, h, l), and an overlapping image of the two fluorescence probes (e, g, k). Abundant punctuate immunofluorescent spots for Cx43 are present in the smooth muscle of E 19–20 rat trachea (d), gradually increasing in the 1-week-old (b) and 3-week-old trachea (l). In contrast, Cx37 spots are not present in the tracheal smooth muscle of the E 19–20 rat (b), are detected only slightly in the 1-week-old (f), but show a considerable increase in the 3-week-old smooth muscle (j). Almost all the Cx37 spots are colocalized with Cx43 in the 1-week-old rat trachea (g). In the 3-week-old rat trachea, some of the Cx37 spots are segregated from the Cx43 spots (k). Transmission images of the same sections are shown in a, e, and i. E epithelium, LP lamina propria, SM smooth muscle. Bars (d, h, l) = 50 μm.
abundantly in the tracheal smooth muscle layer, and were partially colocalized with Cx37. Colocalized spots comprising both Cx37 and Cx43 may indicate gap junction plaques between smooth muscle cells, while those Cx43 spots which were not colocalized with Cx37 may be attributed to gap junction plaques between fibroblastic cells.

We also examined the expression of Cx37 and Cx43 in various smooth muscles. The smooth muscle of effector organs is contracted by the responses to autonomic nerve impulses (Hoffman et al., 1996), or by myogenic activity. These stimulus impulses were conducted via the gap junction. The ductus deferens, urethra, and urinary bladder are organs which are densely innervated by autonomic nerves; however, gap junctions were not detected in these organs (Gabella and Uvelius, 1990). This fact may be consistent with our inability to find any Cx37 or Cx43 in these same organs. Winterhager et al. (1991) reported that Cx43 was present in the pregnant uterus, but we could not detect it in the non-pregnant uterus. The expression of Cx43 in the uterus has been reported to increase just prior to delivery (Orsino et al., 1996). This discrepancy regarding the expression of Cx43 in the uterus may be due to the stage of the uterus examined.

The expression of Cx37 in tracheal smooth muscle cells revealed a gradual increase during postnatal development. In the E20 rat trachea, Cx37 was not detected, although Cx43 already existed. The expression of Cx43 was almost stable; however, Cx37 was detected only after birth, and its expression gradually increased. Most of the Cx37 spots were colocalized with Cx43 spots in 1-week-old rats, but some of the Cx37 spots were segregated from the Cx43 spots in 3-week-old rats. Similarly, the expression of Cx26, Cx31.1, Cx37, and Cx43 in the rat epidermis changed during development (Goliger and Paul, 1994). It is likely that these developmental changes in connexin expression confirm that gap junction-mediated intercellular communication is important for the proper growth, differentiation, and/or the function of cells.

In conclusion, we have shown that Cx37 and Cx43 spots are partially colocalized at the same gap junction plaques connecting the smooth muscle cells of the rat trachea and large arteries. Cx37 was detected after birth in tracheal smooth muscle cells and increased during development. In contrast to Cx37, Cx43 was consistently expressed. Smooth muscle cells in various organs revealed different expression patterns with regard to Cx37 and Cx43. These stage-specific and cell-type-specific expression patterns of different connexins may reflect the functional importance of gap junction channels in smooth muscle cells.

Acknowledgements. We thank Dr. A. KURAOKA for supplying the Cx43 probe, and Dr. H. IIDA for his expert technical advice. The English used in this manuscript was revised by Miss K. MILLER (Royal English Language Center, Fukuoka, Japan).

REFERENCES


Dr. Kyoko Nakamura
Department of Anatomy
Faculty of Medicine
Kyushu University
Fukuoka
812-8582 Japan

中村 哲子
812-8582
福岡市東区馬出3-1-1
九州大学医学部
解剖学第二講座

Tel: +81-92-642-6052
Fax: +81-92-642-6202
E-mail: knaka@ana2.med.kyushu-u.ac.jp