NOTE *Internal Medicine*

Molecular Cloning of Feline Lung Resistance-Related Protein (LRP) cDNA and its Expression in a Feline Lymphoma Cell Line and Adriamycin-Resistant Subline

Kenjiro FUKUSHIMA[1)], Yoshiko OKAI[2)], Shinobu MATSUURA[3)], Hajime TSUJIMOTO[3)] and Yasuyuki ENDO[1)]*

[1)]*Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, Kagoshima University, 1–21–24 Korimoto, Kagoshima 890–0065,* [2)]*Takeda Chemical Industries, Ltd., 2–17–81 Jusohonmachi, Yodogawa-ku, Osaka 532–8686 and* [3)]*Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan*

(Received 16 January 2006/Accepted 17 April 2006)

ABSTRACT. Molecular cloning of feline lung resistance-related protein (LRP) was performed to evaluate the relationship between its expression level and drug resistance against chemotherapeutics. The nucleotide sequence of the coding region of feline *LRP* cDNA was found to be 2670-bp long and to show 84.2–92.6% homology to its human, mouse, and rat counterparts. The expression level of feline *LRP* mRNA was relatively high in lung, jejunum, and colon. An adriamycin (ADM)-resistant feline lymphoma subline, FT-1/ADM, showed a high level of *MDR1* mRNA expression compared with parental FT-1 cells. However, no relationship was observed between the drug-resistant phenotype and the *LRP* mRNA expression level. Although no direct contribution of LRP to the development of the drug-resistant phenotype was observed, further investigation is advisable.
KEY WORDS: feline, LRP, MDR.

J. Vet. Med. Sci. 68(8): 885–890, 2006

Veterinary clinicians in small animal practice often encounter cases exhibiting tumors. Considerable efforts have been made in the field of veterinary oncology to find cures for a number of these malignancies, and as a result, we have many therapeutic options to choose from, including surgical removal, chemotherapy, and radiation therapy. Chemotherapy, one of these options, is generally applied to hematopoietic tumors, such as lymphoma and myeloproliferative disorders, and to some solid tumors that cannot be treated with surgery or radiation therapy. However, there are still some problems in administering chemotherapy against these tumors. One of the major problems with chemotherapy is the induction of multidrug resistance (MDR) in the tumor cells. If MDR is induced, tumor cells are likely to develop a phenotype that is resistant not only to the previously-administered drug but also to other chemotherapeutics-even if they have not been used in the tumor bearing patient and do not have any functional or conformational relationship with the previously-administered drug. The concept of MDR was first proposed by Farber *et al.*, and Kartner *et al.* reported that a P-glycoprotein (P-gp) with a molecular weight of approximately 170 kDa was one of the factors responsible for the induction of MDR [5, 9]. However, a number of recent reports concerning MDR have suggested that P-gp is not the only factor in the development of MDR in tumor cells [2, 4, 19]. These studies revealed that the expression of MDR-associated protein (MRP), lung resistance-related protein (LRP), drug resistance-associated protein (DRP), breast cancer resistance protein (BCRP), and adenosine triphosphate-binding cassette protein (ABCP) were also related to the development of MDR in tumor cells [1, 13, 16, 17, 19]. However, no mechanism other than the MDR gene expression responsible for development of the MDR phenotype in tumor cells has been clarified in the field of small animal veterinary practice.

LRP, one of the factors related to MDR mentioned above, was isolated by Scheper *et al.* in 1993 from a human small cell lung carcinoma cell line showing non-P-gp dependent MDR [19]. LRP was later shown to be a molecule identical to major vault protein and to have a function in the transportation of various substrates between the cytoplasm and the nucleus [11, 18, 20]. Most LRP is located in the cytoplasm and some is located in the nuclear membrane [22]. A correlation between the expression level of LRP and MDR in tumor cells was reported for both solid and hematopoietic tumors, and LRP seemed to induce the MDR-phenotype in tumor cells by trapping chemotherapeutic reagents in the cytoplasm and transporting them out of the nucleus [8]. Furthermore, it has been shown that LRP worked as a prognostic factor for human acute myeloid leukemia [12]. Recently, several veterinary articles focusing on the *MDR1* gene that encodes P-gp have proposed a partial mechanism for the emergence of the MDR-phenotype in tumor cells [3, 14, 23]. However, the structure and function of feline LRP and its ability to induce MDR in tumor cells have yet to be investigated. Thus, we focused on LRP in the present study. Molecular cloning of feline *LRP* cDNA was performed and its expression levels in normal and adriamycin (ADM)-resistant feline lymphoma cell lines were measured.

First, we performed molecular cloning of feline *LRP* cDNA using the RT-PCR method and DNA sequencing. Total RNA was extracted from the spleen of a feline fetus using a commercially available kit (RNeasy Mini, QIAGEN, Hilden, Germany), and then cDNA was synthesized from 1 μg of total RNA using a First Strand cDNA Synthesis Kit (Amersham Biosciences, Piscataway, NJ). Oligonucleotide primers, 5'-TGGGCTTAGGAGTCAC-

* CORRESPONDENCE TO: ENDO, Y., Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, Kagoshima University, 1–21–24 Korimoto, Kagoshima 890–0065, Japan.

CATG-3' (LRP-H-F6, nt 44–63 in human LRP) and 5'-ATGCCCAGAAACTTCCATTG-3' (LRP-H-R6, nt 2775–2756 in human LRP), were designed based on conserved sequences of human, mouse, and rat *LRP* cDNAs [10, 24]. PCR amplification was performed under the following conditions: 1 cycle of pre-denaturation (5 min, 95°C); 30 cycles of denaturation (1 min at 95°C), annealing (1 min at 52°C), and polymerization (1 min at 72°C); and 1 cycle of complete elongation (10 min at 72°C). The amplified DNA fragment was inserted into a pCR2.1 vector (Invitrogen, Carlsbad, CA), and the nucleotide sequence of the inserted DNA fragments was determined by the dideoxy chain termination method (ABI Prism BigDye Primer Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, CA). A 2730-bp long DNA fragment was obtained from RT-PCR using primers LRP-H-F6 and LRP-H-R6. Based on sequencing analysis, this DNA fragment contained a putative coding region that was 2670-bp long and that encoded 890 amino acid residues (Fig. 1). This feline *LRP* cDNA showed strong similarities in its nucleotide sequence to those of the human (89.0%), mouse (92.6%), and rat (84.2%) (Fig. 1). Figure 2 shows the alignment of the predicted amino acid sequence of feline LRP with its human, mouse, and rat homologues. At the amino acid level, feline LRP was shown to be between 90.1 and 92.5% similar to its human, mouse, and rat counterparts. Four cystein residues were conserved at 59, 282, 515 and 572 in the LRPs of all species. Feline LRP was three amino acid residues shorter than human LRP and 29 residues longer than the mouse and rat LRPs at the C-terminus. According to structural analysis using ScanProsite (http://ca.expasy.org/prosite/), feline LRP was shown to possess N-linked glycosylation sites at amino acid residues 24–27 and 453–456, and an EF-hand motif at 131–143. The EF-hand motif is generally known as a calcium-binding domain, which suggests that feline LRP may also have such characteristics and may be involved in the transport of various drugs and substrates between the nucleus and the cytoplasm. These findings suggest that the biological properties of *LRP* gene products may be conserved among species.

Next, we analyzed the expression levels of *LRP* mRNA in normal tissue. Total RNA was extracted from the brain, heart, lung, thymus, liver, spleen, kidney, jejunum, and colon of a cat fetus. Then, cDNA was synthesized as described above. A primer pair 5'-GCCTGACTTCTTCACAGACG-3' (nt 1557–1576 in the sequence of feline *LRP* cDNA obtained in this study) and 5'-TGGGAGTTGGTGGTGATCTC-3' (nt 2006–1987), was used to amplify feline *LRP* mRNA. Sequencing analysis confirmed proper amplification of the derived *LRP* DNA fragment. As an internal control, feline GAPDH cDNA was amplified using a primer pair, 5'-CTCATGACCACAGTCCATGC-3' (nt 514–533 in feline *GAPDH* cDNA, GenBank/EMBL/DDBJ accession number AB038241) and primer 5'-TGAGCTTGACAAAGTGGTCA-3' (nt 925–906). The PCR products were electrophoresed through 2.0% agarose gel and stained with ethidium bromide for visualization. The expression levels of feline *LRP* mRNA were evaluated by band density and compared to that of *GAPDH* mRNA. Graphic data from the stained gel was analyzed by computer using the NIH Image 1.62 software (NIH, Bethesda, MD) as previously reported [25]. As shown in Fig. 3, expression of *LRP* mRNA was observed in a broad range of tissues. Expression of *LRP* mRNA was relatively high in the lung, jejunum, and colon, but was low in the brain and heart. It has been reported previously that organs and cells, such as bronchia, gastro-intestines, and keratinocytes, that are chronically exposed to high concentrations of xenobiotics show a high expression level of LRP [7]. Our results also support these findings. Therefore, it is reasonable to assume that the biological function of feline LRP is the same as in other species.

The relationship between the expression level of the *LRP* gene and the induction of the MDR phenotype was also evaluated using a feline lymphoma cell line, FT-1, and its ADM-resistant subline, FT-1/ADM [14]. FT-1/ADM has been shown to express the *MDR1* gene, which is highly correlated with the development of the MDR phenotype. We also evaluated the expression level of the *MDR1* gene in both the original FT-1 cell line and FT-1/ADM subline using a method described previously [14]. This procedure revealed that the expression level of the *MDR1* gene was significantly higher in the FT-1/ADM subline (relative mRNA expression index against *GAPDH*, 1.99 ± 0.06 [mean ± SD]) than in the parental FT-1 cell line (0.12 ± 0.02) ($p<0.01$, student's t test, Fig. 4). In addition, a dye exclusion test confirmed that the FT-1/ADM subline used in this study survived in culture media with 400 *n*g/m*l* of ADM, while the parental FT-1 cells died in the same medium within two days (data not shown). However, the expression indices for LRP were 0.27 ± 0.02 and 0.26 ± 0.03 in the FT-1 cell line and FT-1/ADMsubline, respectively, and no significant differences were observed ($p=0.74$). This strongly suggests that the MDR-phenotype in the FT-1/ADM subline was mainly induced by high expression of the *MDR1* gene, and not by *LRP*. In other words, P-gp does play a major role in the development of the MDR-phenotype in the FT-1/ADM subline. In this study, we unexpectedly found that there was no relationship between the mRNA expression level of *LRP* and the phenotype of ADM resistance. However, previous reports have shown a relationship between the development of other MDR phenotypes and LRP expression in tumor cells. For instance, the drug resistant subclone of the human mammary gland adenocarcinoma cell line selected by mitoxantrone was shown to overexpress LRP, but not P-gp and MRP, suggesting that LRP is a major factor for the development of the MDR-phenotype [6]. Overexpression of LRP has also been observed in various other tumor cells showing P-gp-independent MDR phenotypes, such as lung cancer, fibrosarcoma, breast cancer, and melanoma [19]. Furthermore, a human myeloma cell line in which MDR1 and LRP were co-expressed was reported to reveal enhanced MDR characteristics compared to parental cells [21]. This finding may

```
                1                                                          73
tgggcttaggagtcaccATGGCAACCGAAGAGTCCATCATCCGCATCCCCCCATACCACTACATCCACGTGCTGGACCAGAACAGCAACG
                  M  A  T  E  E  S  I  I  R  I  P  P  Y  H  Y  I  H  V  L  D  Q  N  S  N
                                                                           163
TGTCCCGCGTGGAGGTCGGGCCAAAGACTTACATCCGGCAGGACAATGAGAGGGTCCTGTTTGCCCCCATGCGCATGGTGACTGTCCCCC
V  S  R  V  E  V  G  P  K  T  Y  I  R  Q  D  N  E  R  V  L  F  A  P  M  R  M  V  T  V  P
                                                                           253
CACGCCACTACTGTACAGTGGCCAACCCGGTGTCCCGGGATCCCCAGGGCTTGGTGCTGTTCGACGTCACAGGGCAAGTACGGCTCCGCC
P  R  H  Y  C  T  V  A  N  P  V  S  R  D  P  Q  G  L  V  L  F  D  V  T  G  Q  V  R  L  R
                                                                           343
ACGCTGACCTAGAGATCCGGCTGGCCCAGGACCCCTTCCCCCTGTACCCAGGGGAGGTGCTGGAAAAGGACATCACTCCACTGCAGGTGG
H  A  D  L  E  I  R  L  A  Q  D  P  F  P  L  Y  P  G  E  V  L  E  K  D  I  T  P  L  Q  V
                                                                           433
TTCTGCCCAACACTGCCCTCCATCTTAAGGCGTTGCTGGATTTTGAGGATAAGAATGGAGAGAAGGTGGTGGCAGGAGATGAGTGGCTAT
V  L  P  N  T  A  L  H  L  K  A  L  L  D  F  E  D  K  N  G  E  K  V  V  A  G  D  E  W  L
                                                                           523
TTGAAGGACCTGGCACATATATCCCCCAGAAGGAGGTGGAGGTCCTGGAGATTATCCAGGCCACGGTCATCAGGCAGAACCAGGCCCTGC
F  E  G  P  G  T  Y  I  P  Q  K  E  V  E  V  L  E  I  I  Q  A  T  V  I  R  Q  N  Q  A  L
                                                                           613
GGCTGAGGGCCCGCAAGGAGTGCTGGGACCGGGACGGCAAGGAGAGGGTGACAGGGGAAGAATGGCTGGTGCGTTCCGTGGGGGCGTATC
R  L  R  A  R  K  E  C  W  D  R  D  G  K  E  R  V  T  G  E  E  W  L  V  R  S  V  G  A  Y
                                                                           703
TCCCGGGAGTGTTTGAGGAGGTTCTGGATTTGGTGGACGCCGTGATCCTCACAGAAAAGACGGCCCTGCACCTCCGGGCTCGGCAGAACT
L  P  G  V  F  E  E  V  L  D  L  V  D  A  V  I  L  T  E  K  T  A  L  H  L  R  A  R  Q  N
                                                                           793
TCCGAGACTTGAGAGGAGTGACCCGTCGCACGGGGGAGGAGTGGCTGGTGACCGTGCAGGACACGGAGGCCCATGTGCCGGACGTCTACG
F  R  D  L  R  G  V  T  R  R  T  G  E  E  W  L  V  T  V  Q  D  T  E  A  H  V  P  D  V  Y
                                                                           883
AGGAGGTGCTGGGGGTCGTGCCCATCACCACCTTGGGCCCCCGCAACTACTGTGTGATTCTCGACCCGGTGGGACCGGATGGCAAGAACC
E  E  V  L  G  V  V  P  I  T  T  L  G  P  R  N  Y  C  V  I  L  D  P  V  G  P  D  G  K  N
                                                                           973
AGCTGGGGCAGAAGCGTGTGGTCAAGGGCGAGAAGTCTTTTTTCCTCCAGCCAGGAGAGAGGCTAGAGCGAGGCATCCAGGATATATATG
Q  L  G  Q  K  R  V  V  K  G  E  K  S  F  F  L  Q  P  G  E  R  L  E  R  G  I  Q  D  I  Y
                                                                          1063
TGCTGTCAGAGCAGCAGGGGCTGCTGCTGAGGGCCCTGCAACCCCTGGAGGAGGGAGAAGAGGAGGGGAAGGTCTCCCACCAGGCTGGGG
V  L  S  E  Q  Q  G  L  L  L  R  A  L  Q  P  L  E  E  G  E  E  E  G  K  V  S  H  Q  A  G
                                                                          1153
ACAACTGGCTCATCCGCGGGCCCCTGGAGTACGTGCCCTCTGCCAAGGTAGAGGTGGTGGAAGAACGTCAGGCCATCCCTCTGGACGAGA
D  N  W  L  I  R  G  P  L  E  Y  V  P  S  A  K  V  E  V  V  E  E  R  Q  A  I  P  L  D  E
                                                                          1243
ATGAGGGCATCTACGTGCAGGATGTCAAGACTGGAAGGGTACGAGCTGTGATTGGAAGCACCTACATGCTGACCCAGGACGAAGTCCTGT
N  E  G  I  Y  V  Q  D  V  K  T  G  R  V  R  A  V  I  G  S  T  Y  M  L  T  Q  D  E  V  L
                                                                          1333
GGGAGAAGGAGCTACCTCCTGGGGTGGAGGAGCTGCTGAACAAGGGGCAGGACCCGCTGGCAGACAGGGGTGTGAAGGAGGTGCCCAAGA
W  E  K  E  L  P  P  G  V  E  E  L  L  N  K  G  Q  D  P  L  A  D  R  G  V  K  E  V  P  K
                                                                          1423
CCCCCCAGCCCTCCCCTCTCCGGAACAAGACCCGCGTGGTCAGCTACCGGGTCCCTCACAACGCTGCCGTGCAGGTGTATGACTACAGAG
T  P  Q  P  S  P  L  R  N  K  T  R  V  V  S  Y  R  V  P  H  N  A  A  V  Q  V  Y  D  Y  R
                                                                          1513
AGAAGAGAGCTCGTGTGGTCTTTGGGCCGGAGCTGGTGTCGCTGGGTCCCGAGGAGCAGTTCACAGTGTTGTCCCTCTCGGCCGGGAGGC
E  K  R  A  R  V  V  F  G  P  E  L  V  S  L  G  P  E  E  Q  F  T  V  L  S  L  S  A  G  R
                                                                          1603
CCAAGCGTCCCCACGCCCGCCGCGCTCTCTGCCTGCTGCTCGGGCCTGACTTCTTCACAGACGTCATCACCATCGAAACAGCAGACCATG
P  K  R  P  H  A  R  R  A  L  C  L  L  L  G  P  D  F  F  T  D  V  I  T  I  E  T  A  D  H
                                                                          1693
CCAGGCTGCAGCTGCAGCTCGCCTACAACTGGCACTTTGAGCTGAGGGACCGGAAGGACCCACAGGAGACAGCCAAGCTCTTCTCAGTGC
A  R  L  Q  L  Q  L  A  Y  N  W  H  F  E  L  R  D  R  K  D  P  Q  E  T  A  K  L  F  S  V
                                                                          1783
CTGACTTCGTGGGTGACGCCTGCAAGGCCATCGCGTCCCGGGTGCGGGGGGCCGTGGCCTCTGTCACCTTCGATGACTTCCATAAGAATT
P  D  F  V  G  D  A  C  K  A  I  A  S  R  V  R  G  A  V  A  S  V  T  F  D  D  F  H  K  N
                                                                          1873
CTGCCCGCATTATTCGCACTGCTGTCTTTGGCTTTGAGACCCCAGAAACCAAGGGGCTCGACGGCAGGGCTCTGCTCCAGCCCCGGGACC
S  A  R  I  I  R  T  A  V  F  G  F  E  T  P  E  T  K  G  L  D  G  R  A  L  L  Q  P  R  D
                                                                          1963
GGGCCGTCTTCCCCCAAAACGGGTTGGTGGTCAGCAGCGTGGACGTGCAGTCGGTGGAGCCCGTGGACCAGAGGACCCGGGACGCCTTAC
R  A  V  F  P  Q  N  G  L  V  V  S  S  V  D  V  Q  S  V  E  P  V  D  Q  R  T  R  D  A  L
                                                                          2053
AGCGCAGCGTCCAACTGGCCATTGAGATCACCACCAACTCCCAGGAGGCAGCTGCCAAGCACGAGGCTCAGAGACTTGAGCAAGAAGCCC
Q  R  S  V  Q  L  A  I  E  I  T  T  N  S  Q  E  A  A  A  K  H  E  A  Q  R  L  E  Q  E  A
                                                                          2143
GGGGCCGGCTTGAGAGGCAGAAGATCTTGGACCAATCCGAAGCTGAAAAGGCTCGCCGCGAACTCTTGGAGCTGGAGGCTCTGAGCACTG
R  G  R  L  E  R  Q  K  I  L  D  Q  S  E  A  E  K  A  R  R  E  L  L  E  L  E  A  L  S  T
                                                                          2233
CCGTGGAGAGCACGGGGACCGCCAAGGCGGAGGCCGAGTCCCGCGCTGAGGCCCTGCGCATCGAGGGAGAAGGCTCCGTGCTACAGGCCA
A  V  E  S  T  G  T  A  K  A  E  A  E  S  R  A  E  A  L  R  I  E  G  E  G  S  V  L  Q  A
                                                                          2323
AGTTGAAAGCCGAGGCCTTGGCCATTGAGACGGAGGCTGAGCTCCAACGGGTACGGAAAGTGCGAGAACTAGAATTGGTCTATGCCCGGG
K  L  K  A  E  A  L  A  I  E  T  E  A  E  L  Q  R  V  R  K  V  R  E  L  E  L  V  Y  A  R
                                                                          2413
CCCAGCTGGAGCTGGAGGTGAGCAAGGCCCAGCAGCTGGCGGAGGTGGAGGTGAAGAAGTTCAAGCAGATGACGGAGGCCCTGGGCCCCA
A  Q  L  E  L  E  V  S  K  A  Q  Q  L  A  E  V  E  V  K  K  F  K  Q  M  T  E  A  L  G  P
                                                                          2503
GCACCATCAGGGACCTTGCTGTGGCTGGGCCAGAGATGCAGGTGAAACTGCTCCAGTCCCTGGGCCTGAAATCAACGCTCATCACCGATG
S  T  I  R  D  L  A  V  A  G  P  E  M  Q  V  R  L  L  Q  S  L  G  L  K  S  T  L  I  T  D
                                                                          2593
GTTCCACCCCCATCAACCTCTTCAACACAGCCTTGGGTCTCCTGGGGCTGGGGTCGGAGGCCCAGCCCCCAACCAAAAAGGCAGCCGGCA
G  S  T  P  I  N  L  F  N  T  A  L  G  L  L  G  L  G  S  E  A  Q  P  P  T  K  K  A  A  G
                                                                     2670
TGCCCAGCCCCCAAGAGGGCTTGCTTCTCCGGTCCCCTCCTGCCCCTCAGTCTCTTGGAGACAACCATGTTGTCCCTtagcgccgtgctg
M  P  S  P  Q  E  G  L  L  L  R  S  P  P  A  P  Q  S  L  G  D  N  H  V  V  P  *

caaccgacacaatggaagtttctgggcat
```

Fig. 1. Nucleotide and deduced amino acid sequences of feline *LRP* cDNA. The locations of primers are underlined. The accession number of the nucleotide sequence of feline *LRP* cDNA is AB244733 in GenBank/EMBL/DDBJ.

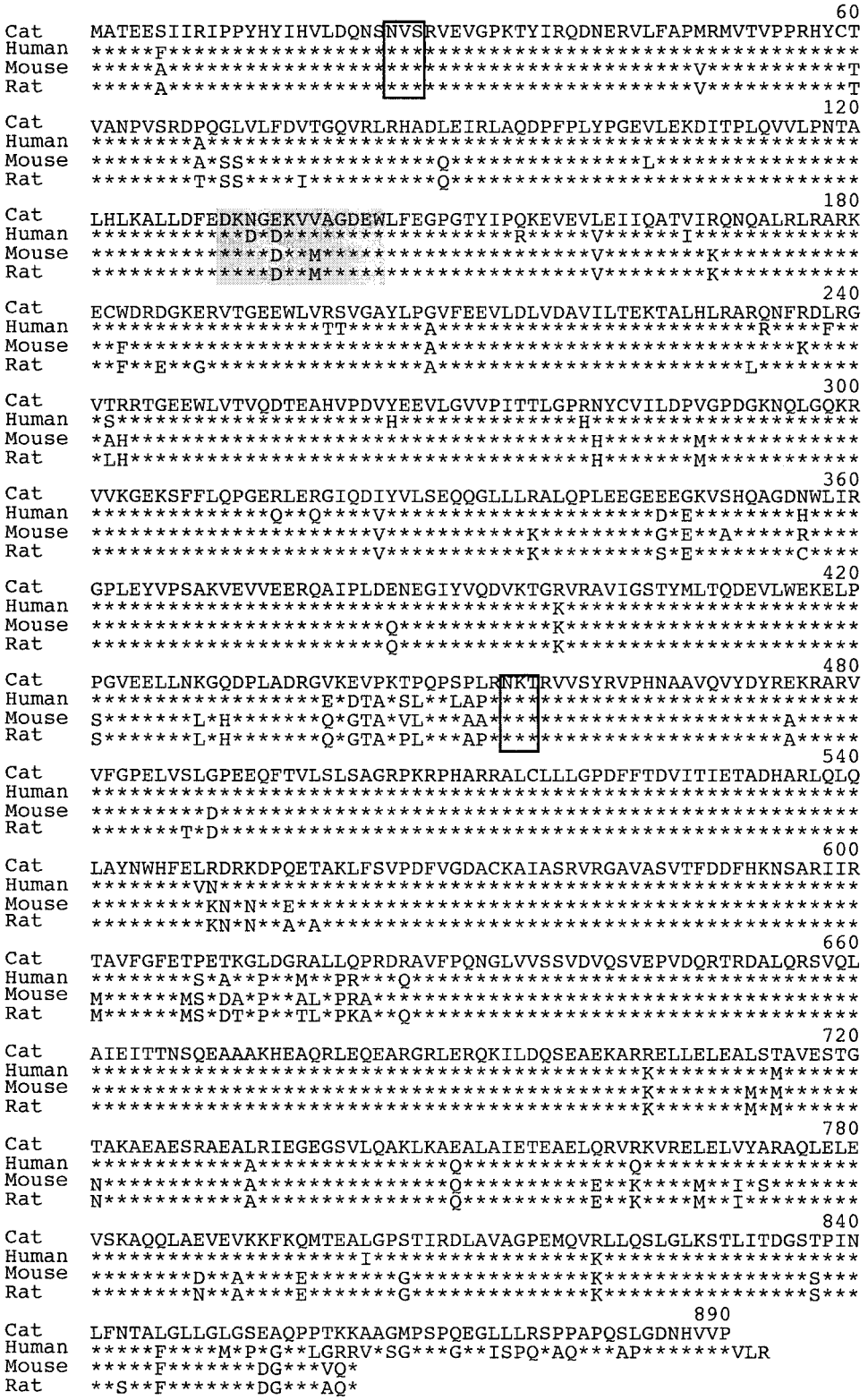

Fig. 2. Comparison of the amino acid sequences of feline LRP with their human, mouse, and rat counterparts. Identical amino acid residues among species are shown as asterisks. Open and shaded boxes indicate the N-linked glycosylation site and EF-hand motif, respectively.

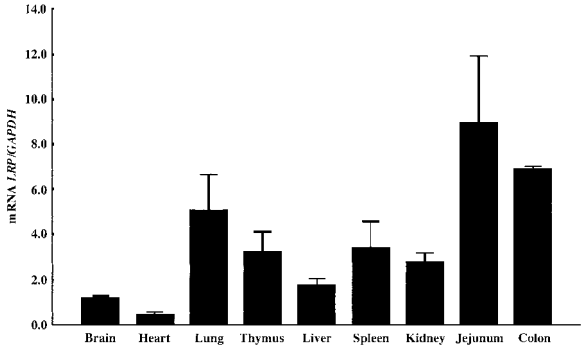

Fig. 3. Comparison of expression of *LRP* mRNAs in normal tissue derived from a feline fetus. All data are depicted as mean relative indices against *GAPDH* mRNA level with standard deviation from three independent analyses.

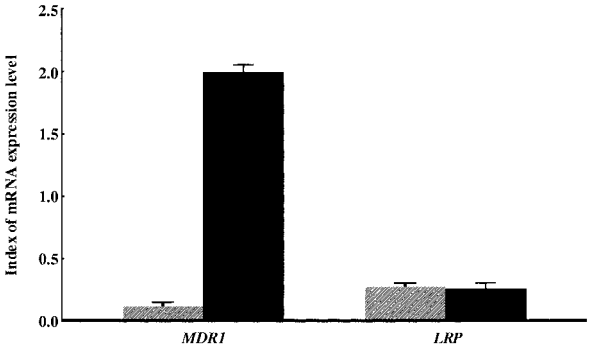

Fig. 4. Comparisons of expression levels of *MDR1* and *LRP* mRNAs in the FT-1 cell line and its FT-1/ADM subline. Grey and black boxes indicate mRNA expression indices in FT-1 and FT-1/ADM cells, respectively. All data are depicted as mean values with standard deviation from three independent analyses.

mean that LRP exerts synergistic and/or additive effects in the development of the MDR-phenotype in tumor cells. While many reports of this kind, indicating the contribution of LRP to MDR, have been published, the absence of a relationship between LRP expression and the MDR-phenotype in human breast cancer has also been reported [15]. As described above, there are still inconsistencies in the observed relationships between LRP and the development of the MDR-phenotype. LRP expression might be dependent on the type of cell and/or the type of drugs used. Therefore, further investigations are necessary to determine the contribution of LRP to the development of MDR-phenotype in tumor cells by making use of a range of different types of cell lines and clinical tumor samples.

In this study, we determined the nucleotide and putative amino acid sequences of feline LRP with the aim of investigating its possible importance in the emergence of the acquired MDR-phenotype. Although we did not find any evidence of a relationship between ADM resistance and *LRP* gene expression level in the feline cell line, the role of LRP in relation to MDR might become clearer in the field of small animal practice if we were to evaluate *LRP* expression levels in a range of tumor cases that have undergone chemotherapy and exhibited MDR. This work was supported by grants from the Japan Society for the Promotion of Science.

REFERENCES

1. Allikmets, R., Schriml, L. M., Hutchinson, A., Romano-Spica, V. and Dean, M. 1998. *Cancer Res.* **58**: 5337–5339.
2. Cole, S. P., Bhardwaj, G., Gerlach J. H,. Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M. and Deeley, R. G. 1992. *Science* **258**: 1650–1654.
3. Culmsee, K., Gruber, A. D., von Samson-Himmelstjerna, G. and Nolte, I. 2004. *Res. Vet. Sci.* **77**: 223–229.
4. Doyle, L. A., Yang, W., Abruzzo, L. V., Krogmann, T., Gao, Y., Rishi, A. K. and Ross, D. D. 1998. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 15665–15670.
5. Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F. and Wolff, J. A. 1948. *New Engl. J. Med.* **238**: 787–793.
6. Futscher, B.W., Abbaszadegan, M. R., Domann, F. and Dalton, W.S. 1994. *Biochem. Pharmacol.* **47**: 1601–1604.
7. Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Giaccone, G., Broxterman, H. J., Meijer, C. J., van der Valk, P. and Scheper, R. J. 1996. *Am. J. Pathol.* **148**: 877–887.
8. Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Shoemaker, R. H., Rome, L. H. and Scheper, R. J. 1996. *Cytotechnology* **19**: 191–197.
9. Kartner, N., Riordan, J. R. and Ling, V. 1983. *Science* **221**: 1285–1288.
10. Kickhoefer, V. A. and Rome, L. H. 1994. *Gene* **151:** 257–260.
11. Kitazono, M., Sumizawa, T., Takebayashi, Y., Chen, Z.H., Furukawa, T., Nagayama, S., Tani, A., Takao, S., Aikou, T. and Akiyama, S. 1999. *J. Natl. Cancer Inst.* **91**: 1647–1653.
12. List, A. F., Spier, C. S., Abbaszadegan, M., Grogan, T. M., Greer, J. P., Wolff, S. N., Scheper, R. J. and Dalton, W. S. 1993. *Blood* **82**: (*Suppl.* 1) 443a.
13. Marquardt, D., McCrone, S. and Center, M. S. 1990. *Cancer Res.* **50**: 1426–1430.
14. Okai, Y., Nakamura, N., Matsushiro, H., Kato, H., Setoguchi, A., Yazawa, M., Okuda, M., Watari, T., Hasegawa, A. and Tsujimoto, H. 2000. *Am. J. Vet. Res.* **61**: 1122–1127.
15. Pohl, G., Filipits, M., Suchomel, R. W., Stranzl, T., Depisch, D. and Pirker, R. 1999. *Anticancer Res.* **19**: 5051–5055.
16. Ramachandran, C., You, W. and Krishan, A. 1998. *Proc. Am. Assoc. Cancer Res.* **39**: 557.
17. Ross, D. D., Gao, Y., Yang, W., Leszyk, J., Shively, J. and Doyle, L. A. 1997. *Cancer Res.* **57**: 5460–5464.
18. Scheffer, G. L., Schroeijers, A. B., Izquierdo, M. A., Wiemer, E. A. C. and Scheper, R. J. 2000. *Curr. Opin. Oncol.* **12**: 550–556.
19. Scheper, R.J., Broxterman, H. J., Scheffer, G. L., Kaaijk, P., Dalton, W. S., van Heijningen, T. H. M., van Kalken, C. K., Slovak, M. L., de Vries, E. G. E., van der Valk, P., Meijer, C. J. L. M. and Oinedo, H. M. 1993. *Cancer Res.* **53**: 1475–1479.
20. Scheffer, G. L., Wijngaard, P. L. J., Flens, M. J., Izquierdo, M. A., Slovak, M. L., Pinedo, H. M., Meijer, C. J., Clevers, H. C. and Scheper R. J. 1995. *Nat. Med.* **1**: 578–582.
21. Shao, Y., de Giuli, R., Wyler, B. and Lehnert, M. 1995. *Proc. Am. Assoc. Cancer Res.* **36**: 337.
22. Slesina, M., Inman, E. M., Rome, L. H. and Volknandt, W. 2005. *Cell Tissue Res.* **321**: 97–104.
23. Steingold, S. F., Sharp, N. J., McGahan, M. C., Hughes, C. S.,

Dunn, S. E. and Page, R. L. 1998. *Anticancer Res.* **18**: 393–400.
24. Strausberg, R. L., Feingold, E. A., Grouse, L. H. *et al*. 2002. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 16899–16903.
25. Takahashi, M., Une, R., Fukushima, K., Fujiki, M., Misumi, K., Miyoshi, N., Endo, Y., Oishi, A. and Akuzawa. M. 2005. *J. Vet. Med. Sci.* **67**: 837–841.