Expression and Subcellular Localization of E-Cadherin, α -Catenin, and β -Catenin in 8 Feline Mammary Tumor Cell Lines

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(Received 16 December 2005/Accepted 30 March 2007)

ABSTRACT. Protein expression and subcellular localization of E-cadherin, α -catenin, and β -catenin in 8 feline mammary tumor cell lines were examined by western blot analysis and fluorescence immunocytochemistry. A low E-cadherin expression was observed in FNN-m cells. Furthermore, compared to other cell lines, two E-cadherin bands existed in FMC-p1 cells and were localized in the perinuclear region; distinct radial lines were observed in the cytoplasm. A low α -catenin expression was observed in FON-m cells, but there were no apparent abnormalities in its localization. In contrast, similar levels of β -catenin expression and cytoplasmic localization were observed in all cell lines.

KEY WORDS: α - and β -catenin, E-cadherin, feline mammary tumor.

J. Vet. Med. Sci. 69(8): 831–834, 2007

Cellular adhesion molecules are important for maintaining tissue structure and cell polarity, and are involved in cell movement and proliferation [13]. E-cadherin associates with a group of intracellular proteins termed catenins. α -Catenin serves as a bridge between E-cadherin and β -catenin, both of which connect with the microfilament cytoskeleton [20]. Disruption of normal cell-cell adhesion by alterations in cadherin-catenin molecules may contribute to enhanced migration and proliferation of tumor cells, thereby leading to invasion and metastasis [13].

In human and experimental animal models, loss of E-cadherin expression was reported to cause cell migration or metastasis of tumor cells in breast cancers [11], gastric cancers [2], colorectal cancers [9], and prostate cancers [4]. Along with abnormalities of E-cadherin, the relationship between the disruption of the cell adhesion system and α - and β -catenin expressions was also reported in these cancers [7, 8, 17]. In addition to cell adhesion, β -catenin plays a role in the Wnt signaling cascade that regulates many cellular processes, including cell fate decisions and cell proliferation [6]. On the other hand, mutations in β -catenin or other Wnt pathway-related components result in β -catenin accumulation in the nucleus [8]. It has been reported that aberrant Wnt signaling is associated with breast [6], colorectal [3], and pancreatic intraepithelial cancers [1].

In cats, mammary tumors are the third common neoplasms, following skin tumors and lymphomas, and account for 12% of all tumors and 17% of the tumors in queens [15]. More than 80% of feline mammary tumors showed considerable malignancy along with rapid progression and

metastasis at an early stage [12]. Since feline mammary tumors have malignant properties, investigating their carcinogenesis, tumor progression mechanisms, and the expressions of tumor related molecules is extremely essential. The purpose of this study was to investigate the expression and subcellular localization of E-cadherin, α -catenin, and β -catenin in 8 feline mammary tumor cell lines in order to evaluate the abnormalities of these cell adhesion molecules.

In the present study, we used 8 feline mammary tumor cell lines, FKN-p, FMC-p1, FMC-p2, FMC-m, FNN-m, FON-p, FON-m, and FYM-p [21]. Of these, 5 were established from a primary lesion, and 3 were established from a metastatic lesion in feline patients bearing spontaneous mammary tumors. Capital letters such as “FKN” indicate identical patients, and small letters such as “p” and “m”, indicate primary and metastatic lesions, respectively. The cells were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum, 100 mg/ml streptomycin, and 100 IU/ml penicillin (Sigma, St Louis, MO, U.S.A.); they were incubated at 37°C in a humidified atmosphere of 5% CO₂. Antibodies against E-cadherin, α -catenin, and β -catenin were obtained from BD-Transduction laboratories (Lexington, KY, U.S.A.) and used as primary antibodies in this study. The antibody against tubulins (NeoMaker, Fremont, CA, U.S.A.) was used as an internal control. Feline mammary tumor cells under sparse conditions were lysed in RIPA buffer (10 mM Tris-HCl, 1% NP40, 0.1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 1 mM EDTA, 10 μ g/ml aprotinin, 0.1 mM Na₂MoO₄, 2 mM Na₃VO₄, 1 mM PMSF, 10 mM NaF, 25 mM sodium β -glycerophosphate, 10 mM sodium pyrophosphoric acid and 1 mM EGTA). Ten micrograms of protein lysates was resolved by 10% SDS-PAGE and blotted onto a polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences, Little Chalfont, UK),

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Fig. 1. The expression of E-cadherin, α -catenin, and β -catenin in 8 feline mammary tumor cell lines. The E-cadherin and α -catenin expression was low in the FNN-m and FON-m cells, respectively. Moreover, 2 bands of E-cadherin existed in the FMC-p1 cells, wherein the major signal was slightly lower than those of other cells. Tubulin indicated the quantity of protein in each cell.

and then preincubated with PBST buffer containing 5% nonfat milk. The blots were incubated with appropriate primary antibodies for 2 hr at room temperature (RT). Subsequently, they were incubated with horseradish phosphatase-conjugated antibodies against rabbit Ig (Amersham Biosciences) or mouse Ig (Amersham Biosciences) for 1 hr at RT. The signal was detected with ECL Plus Western Blotting Detection system (Amersham Biosciences). The protein expressions of E-cadherin, α -catenin, and β -catenin are shown in Fig. 1. Distinct E-cadherin expression was observed in the FKN-p, FMC-p2, FMC-m, FON-p, FON-m, and FYM-p cells, while that in the FNN-m cells, which were derived from metastatic pleural effusion, was lower. As compared with the 7 other cell lines, FMC-p1 cells showed two E-cadherin bands. The major signal was slightly lower in the FMC-p1 cells, suggesting that the major E-cadherin in the FMC-p1 cells might be smaller than the original protein. Among the 8 feline mammary tumor cell lines examined, 7 showed distinct signals for α -catenin, whereas lower expression was observed in the FON-m cells, which were established from the metastatic lesion of the lymph node. In contrast, a high level of β -catenin expression was detected in all 8 feline mammary tumor cell lines.

For further investigation of the relationship between the difference in expression pattern and cellular localization, we investigated the subcellular localization of E-cadherin, α -catenin, and β -catenin proteins by fluorescence immunocytochemistry according to the recommended protocol of Zenon R Alexia Fluor IgG labeling kit (Invitrogen, San Diego, CA, U.S.A.). The primary antibodies were labeled with Alexia Fluor 448 or 562 according to the protocol for the Zenon Alexia Fluor labeling kit (Invitrogen). All 8 feline mammary tumor cell lines were cultured on Lab-Tek II chamber slides (Nalge Nunc, NY, U.S.A.) to subconfluent growth and fixed in 4% paraformaldehyde solution in PBS

for 15 min at RT. After washing with PBS, the cells were permeabilized by incubation in 0.1% TritonX-100/PBS for 5 min at RT, followed by incubation with normal goat serum for 30 min at RT, and then with fluorescence-labeled primary antibodies for 60 min at RT. The cells were washed, refixed with 4% paraformaldehyde/PBS, and incubated with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) for 5 min. The products were mounted with Aqua Poly/Mount (Polysciences, PA, U.S.A.), and microscopic examination was carried out under fluorescent light (OLYMPUS, Tokyo, Japan). Cell image acquisition, processing and analysis were performed using MetaMorph Imaging Systems (Molecular Devices, CA, U.S.A.).

The merged figures in the picture show the localization of E-cadherin and β -catenin, following nuclear and cytoplasmic staining (Fig. 2. D, H). In the FON-p cells, localization of E-cadherin and β -catenin was almost similar, and they showed uniform distribution in the cytoplasm (Fig. 2 A-D); similar localization patterns were observed in the other cell lines (data not shown). In FMC-p1 cells, however, the intracellular localization of E-cadherin was strongly observed in the perinuclear region, and distinct radial lines were also detected in the cytoplasm (Fig. 2E). E-cadherin and α -catenin were uniformly localized in the cytoplasm of each cell line (data not shown). We could not find any characteristic patterns such as nuclear localization of β -catenin in all 8 feline cell lines (Fig. 2B, F), although distinct expression of β -catenin was determined by western blot analysis (Fig. 1).

In this study, we examined the protein expression and subcellular localization of E-cadherin, α -catenin, and β -catenin in 8 feline mammary tumor cell lines. Among 5 cell lines derived from the primary lesion of feline mammary tumors, E-cadherin in the FMC-p1 cells had an apparently small molecular weight, though similar levels of E-cadherin expression were observed (Fig. 1). Furthermore, its subcellular localization was not observed in the cellular membrane but in the perinuclear region (Fig. 2). These results suggested that this abnormal E-cadherin might have resulted from deletion or mutation of the protein structure. In the studies on E-cadherin abnormalities, partial deletion of E-cadherin was seldom reported, although loss or decreased expression has been reported in various tumors [1, 8, 16, 20]. These findings indicated that exploring the cause of protein deletion of E-cadherin might provide interesting insights into novel systems of E-cadherin dysfunction in tumor cells.

FMC-p1 and -p2 were established from the same lesion of the same patient, but the level and size of E-cadherin expression was different between the two. Although the cause of different E-cadherin expression pattern was unclear, it might be meaningful to compare genomic nucleotide mutations, exon-intron structure, and alteration of gene splicing. In addition, these results would contribute to reveal the molecular mechanism of deletion of E-cadherin protein in FMC-p1 cells.

In 3 cell lines established from the metastatic lesion, a prominent decrease in E-cadherin expression was observed

Fig. 2. The subcellular localization of E-cadherin and β -catenin in the FON-p and FMC-p1 cells based on immunocytochemical analysis. The merged figures in the picture show the findings of both cytoplasmic and nuclear staining of E-cadherin and β -catenin (D, H). In the FMC-p1 cells, subcellular localization of E-cadherin was strongly seen in the perinuclear region and distinct radial lines were observed in the cytoplasm (E). In other cells, the localization of E-cadherin and β -catenin was almost similar, and they were uniformly distributed in the cytoplasm of each feline cell (A-D).

in FNN-m cells. Since decreased E-cadherin expression is thought to be related to metastasis, it might be an useful model to analyze the role of E-cadherin for metastasis of feline mammary tumors. In human gastric and breast cancers, decreased E-cadherin expression was reported to be a consequence of frequent inactivating mutations in the genome of the E-cadherin gene [18]. Investigation on the inactivating mutations in feline mammary tumors would lead to clarify the mechanism of E-cadherin expression.

Among the cells established from the primary and metastatic lesions of the same patients, a reduced α -catenin expression was observed in FON-m cells, which was obtained from the metastatic regional lymph node [21]. Loss of α -catenin expression was studied in the lung and prostate cancer cells [10]. Reduction in α -catenin expression was reported to have a greater correlation with the collapse of cell-cell adhesion than with E-cadherin expression in human esophageal and prostate cancers [16]. In the malignant transformation step of FON-m cell line, decreased α -catenin expression might contribute to the acquisition of malignant properties.

This paper described the expressions of α - and β -catenin in feline mammary tumor cell lines. Several studies reported the expression of E-cadherin in canine [14] and feline [5] mammary tumor tissues, and canine mammary tumor cell lines [19]. According to these previous studies, levels of E-cadherin expression seemed to reduce depending on the increase in malignancy of the mammary tumors, however we could detect the reduction in only one cell line, probably due to the limited number of cell lines. On the contrary, immunocytochemical analysis in the study demonstrated the localization of E-cadherin to be in the membrane

and cytoplasm. The similar localization patterns were not found in the normal feline mammary tissues, but in the solid tumors and carcinomas [5]. This may indicate that the E-cadherin localization may related to the malignant transformation of feline mammary tumors. Further analysis will be needed to clarify the relationship between the metastatic characterization and E-cadherin and catenins.

In conclusion, reduced E-cadherin and α -catenin expression was detected in one of the 8 feline mammary tumor cell lines. In addition, we observed an apparently small E-cadherin protein with a different subcellular localization. However, although we showed distinct β -catenin expression, excessive nuclear localization was not seen in all 8 feline mammary tumor cell lines in the present study. It might be important to determine the cause of decreased expression in E-cadherin and α -catenin, and of deleted protein translation in E-cadherin. This will also help in revealing the frequency of abnormal E-cadherin-catenin expression in spontaneous feline mammary tumors.

ACKNOWLEDGEMENTS. This work was partially supported by a Grant-in-Aid No. 15208030 and a Grant-in-Aid for Scientific Research on Priority Areas "Comprehensive Genomics" from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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