Expression of Fat2 mRNA in Canine Skin Tumors

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Abstract: Fat cadherins are huge proteins that contain 34 cadherin repeats. Recent studies showed that human Fat2 was expressed in squamous cell carcinoma and mammalian fat cadherins related to tumor. It is possible to speculate that Fat2 relate skin tumors. Canine skin tumor is one of the most common diseases and some skin tumors like melanoma are devastating. Therefore, many kinds of investigation about skin tumor are necessary for a certain and prompt diagnosis and prediction. We investigated the expression of Fat1 and Fat2 mRNA in canine skin biopsies by RT-PCR. Samples of skin tissue were obtained from five healthy dogs and skin tumors of nine dogs from private veterinary hospital (Kitasuma Animal Hospital and Matsuoka Animal Hospital). Fat2 mRNA was not expressed in normal canine skin. However, we found that canine Fat2 mRNA was expressed in six of nine (67%) canine skin tumors. On the other hand, canine Fat1 mRNA was not detected in canine normal skin and skin tumors. These results indicate that Fat2 might relate to skin tumors in dogs. The further study of Fat2 in dog might be important for clarifying the mechanism of skin tumors and developing the treatment.

Key words: Cadherin, canine, Fat2, skin, skin tumor, Japan

INTRODUCTION

The Ca (2+)−dependent cell−cell adhesion molecules, termed cadherins are components of intercellular adhesive junctions of epithelial cells. Various human or canine cadherins are expressed in skin and play a crucial role in the formation and maintenance of epithelial barriers and are involved in skin diseases (Takeuchi et al., 2002; Yabuzoe et al., 2009).

Fat cadherins are huge proteins that contain 34 cadherin repeats and that form a distinct subfamily of the cadherin superfamily (Tanoue and Takeichi, 2004). Four kinds of Fat cadherin, Fat1−Fat4 have been identified. Fat was identified as a tumor suppressor protein in Drosophila (Mahoney et al., 1991). It has been reported that Fat1 and Fat2 are expressed in squamous cell carcinoma (Tanoue and Takeichi, 2004; Matsu et al., 2007). In addition, some studies relating to tumor have been reported in mammal Fat cadherins (Qi et al., 2009).

Canine skin tumor is one of the most common diseases. Cutaneous neoplasms were diagnosed in 22.8% of dogs based on the retrospective study (Villamil et al., 2011). Lipoma, adenoma and mast cell tumor are the most common skin tumor types (Villamil et al., 2011) and some kinds of skin tumor like melanoma are devastating. Therefore, diagnosis is crucial for their life. Many studies about diagnostic marker, especially about melanoma and mast cell tumor have been reported such as S-100 protein for melanoma (Sandusky et al., 1985) and c-kit protein for mast cell tumor (Passantino et al., 2008). However, there is no single diagnostic technique capable of differentiating a benign tumor from devastating neoplasms or predicting survival time. Therefore, a further investigation about skin tumor of dog is necessary for a certain and prompt diagnosis and prediction.

The purpose of this study was to analyze the expression of Fat1 and Fat2 mRNA in canine skin because human Fat2 expressed in skin is possible to speculate the relationship to skin tumors. We found for the first time interesting results that canine Fat2 mRNA expressed in only skin tumors but not in normal skin.

MATERIALS AND METHODS

Preparation of cDNA: Animals were housed in the laboratory research facility at Osaka Prefecture University and the proper management of animals are carried out under the principles and guidelines approved by Osaka Prefecture University. Mouse (C57/B6L) and rat (Wistar/ST rat) skin specimens were split from root of the
Table 1: Characteristics of canine patients with skin tumors

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Site</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>Acute/chronic</th>
<th>Benign/malignant</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillon</td>
<td>Anal</td>
<td>Papilloma</td>
<td>11</td>
<td>Neutered male</td>
<td>Acute</td>
<td>Unknown</td>
<td>No recurrence</td>
</tr>
<tr>
<td>Mongrel</td>
<td>Chest</td>
<td>Hemangiopericytoma</td>
<td>11</td>
<td>Neutered female</td>
<td>Chronic</td>
<td>Malignant</td>
<td>Recurrence</td>
</tr>
<tr>
<td>Sheltie</td>
<td>Cheek</td>
<td>Trichoepithelioma</td>
<td>10</td>
<td>Neutered female</td>
<td>Acute</td>
<td>Benign</td>
<td>No recurrence</td>
</tr>
<tr>
<td>Miniature dachshund</td>
<td>Labia oris</td>
<td>Papilloma</td>
<td>12</td>
<td>Neutered female</td>
<td>Acute</td>
<td>Unknown</td>
<td>No recurrence</td>
</tr>
<tr>
<td>Shih Tzu</td>
<td>Chest</td>
<td>Lipoma</td>
<td>Unknown</td>
<td>Neutered male</td>
<td>Unknown</td>
<td>Benign</td>
<td>No recurrence</td>
</tr>
<tr>
<td>Kishu-inu</td>
<td>Mandible</td>
<td>Epithelioma</td>
<td>10</td>
<td>Neutered female</td>
<td>Acute</td>
<td>Benign</td>
<td>No recurrence</td>
</tr>
<tr>
<td>Miniature dachshund</td>
<td></td>
<td>Epithelioma</td>
<td>6</td>
<td>Neutered female</td>
<td>Acute</td>
<td>Unknown</td>
<td>No recurrence</td>
</tr>
<tr>
<td>Mongrel</td>
<td>Mammary gland</td>
<td>Mammary gland adenocarcina</td>
<td>12</td>
<td>Neutered female</td>
<td>Chronic</td>
<td>Malignant</td>
<td>Unknown</td>
</tr>
<tr>
<td>Shepherd</td>
<td>Ear</td>
<td>Trichoepithelioma</td>
<td>8</td>
<td>Female</td>
<td>Acute</td>
<td>Benign</td>
<td>Death</td>
</tr>
</tbody>
</table>

Table 2: Oligonucleotide primers for PCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer</th>
<th>Sequence</th>
<th>Size</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Mouse Fat1</td>
<td>Forward</td>
<td>5'-aacacctgcctcgcatctgctc-3'</td>
<td>471 bp</td>
<td>NM_001081286</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>5'-gtggcttcgctacgagct-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse Fat2</td>
<td>Forward</td>
<td>5'-gcagcttcgctcctacattta-3'</td>
<td>365 bp</td>
<td>NM_001029988.2</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-tctgcactcccttactgca-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse E-cadherin</td>
<td>Forward</td>
<td>5'-aactggggaggaacagact-3'</td>
<td>324 bp</td>
<td>NM_009864.2</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-cgagagctgacgctttcag-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse β-actin</td>
<td>Forward</td>
<td>5'-gcctgcgcggtgggtctctc-3'</td>
<td>487 bp</td>
<td>NM_009860.2</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>5'-cagatgctgaggttttctgc-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat Fat1</td>
<td>Forward</td>
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<td>403 bp</td>
<td>NM_031819.1</td>
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<tr>
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<td>Reverse</td>
<td>5'-actctgagctctagcgttcag-3'</td>
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<td></td>
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<tr>
<td>Rat Fat2</td>
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<td>437 bp</td>
<td>NM_022954</td>
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<td>Reverse</td>
<td>5'-aggatgccagctgctgattc-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat E-cadherin</td>
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<td>354 bp</td>
<td>NM_031341.4</td>
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<td>Reverse</td>
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<td></td>
<td></td>
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<tr>
<td>Rat GAPDH</td>
<td>Forward</td>
<td>5'-aagcttgggcctgcatcgc-3'</td>
<td>725 bp</td>
<td>NM_017098.3</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
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<tr>
<td>canine Fat1</td>
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<td>458 bp</td>
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<tr>
<td>canine Fat2</td>
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<td>408 bp</td>
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<tr>
<td>canine E-cadherin</td>
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<td>442 bp</td>
<td>XM_536807.2</td>
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<td></td>
<td>Reverse</td>
<td>5'-gggttatgctgctgatttc-3'</td>
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<td></td>
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<tr>
<td>canine GAPDH</td>
<td>Forward</td>
<td>5'-tcagctgctggtgctgctgctgt-3'</td>
<td>409 bp</td>
<td>XM_845086</td>
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<td></td>
<td>Reverse</td>
<td>5'-gggttatgctgctgatttc-3'</td>
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</table>

tail using fine forceps and then incubated in 8 mg mL⁻¹ dispase at 4°C overnight to allow separation of epidermis and dermis (Longley et al., 1991). Epidermal sheets were carefully peeled away from dermis. On the other hand, we could not obtain canine skin sample of enough quantity to separate its epidermis. Therefore canine skin biopsies were excised and immediately submerged in RNA stabilization solution, RNAlater (Ambion, Carlsbad, CA). The murine epidermal cell line Pam 212 was generously gifted from Dr. Stuart Yuspa, National Institutes of Health, Bethesda, MD. Total RNA was extracted using RNeasy Mini kit (QIAGEN, Valencia, CA) and first-strand cDNAs were generated using Omniscript RT kit (QIAGEN) with oligo dT primers (Invitrogen, Chiba, Japan).

Biopsies from skin tumors in dogs were obtained from private veterinary hospital between March 16, 2010 and November 9, 2010 based on owner’s acceptance in writing. The biopsies were obtained by mastectomy or block dissection from nine dogs of different breeds (mean age 10 years). Animals had not received any treatment including chemotherapy and radiotherapy, prior to surgery. All specimens were immediately immersed in an RNAlater solution and stored at 4°C until further processing. Histological analysis was performed to ensure that the tissues were either malignant or normal (Table 1).

**RT-PCR:** It was performed RT-PCR using specific primers for Fat1, Fat2, E-cadherin and GAPDH/β-actin (Table 2). The PCR reaction with Taq Master Mix (QIAGEN) was proceeded as follows: denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, hybridization at 60°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. The PCR products were separated on 2% agarose gels and stained with ethidium bromide.

**RESULTS AND DISCUSSION**

At first, the expressions of Fat2 mRNA in normal canine skin (normal female Collie, the same sample as N1), normal mouse epidermis, normal rat epidermis and the murine epidermal cell line Pam 212 were analyzed. The GAPDH and E-cadherin bands were detected as a control. As shown in Fig. 1, Fat1 and Fat2 mRNA were not
mouse and rat epidermis and Pam 212 cells like human
epidermis (Matsui et al., 2007) (Fig. 1). It has been already
reported that Fat1 is expressed in mouse skin (Tanoue and
Takeichi, 2005). We showed for the first time that mouse
and rat epidermis expressed Fat2 mRNA. Human Fat2 was
expressed in Squamous Cell Carcinoma (SCC) in addition
to normal skin (Matsui et al., 2007). Next, we further
investigated the expression of Fat2 mRNA in canine skin
tissues. Samples of skin tissue were obtained from five
healthy dogs and skin tumors of nine dogs (Table 1). Fat2
mRNA expression was restricted to the tumors being
found in six (No.1-5 and 7) of nine (67%) canine skin
tumors but not in any normal canine skin (Fig. 2). We
compared Fat2 mRNA expression in the tumor lesion and
the normal site in same dog which had skin tumor. Fat2
mRNA was not detected in normal skin in other dogs
which have skin tumor. Fat1 mRNA was not detected in
skin tumors of all dogs which we examined.

According to various reports, about 30-45% of all
canine tumors are known to occur in the skin and
subcutaneous tissues (Muller et al., 1983). The incidence
of skin tumors is known to be high at old dogs, female
dogs and dogs of particular breeds (Muller et al., 1983).
In the present study, Fat2 mRNA was expressed in skin
tumors of some dogs but in not all one. It is reported
previously that Fat is a tumor suppressor gene and
knockdown of human Fat2 tended to promote epidermal
cell proliferation (Matsui et al., 2008). These results
indicate that the expression of Fat2 in canine skin might
relate to cell proliferation. Although, it is necessary to
study the immunohistochemical detection of Fat2 protein
in canine normal skin and skin tumors using specific
antibody, canine Fat2 antibody is unavailable in markets
unfortunately. Furthermore, there are some tendencies for
Fat2 mRNA to be expressed in skin tumors of old
dogs (4 dogs is >10 years) (Table 1). There is no
relationship between Fat2 expression and acuteness,
malignancy and prognosis of canine skin tumors
(Table 1). However, further studies of Fat2 are necessary
to determine the relationship to them. Fat1 mRNA was
expressed in mouse epidermis, rat epidermis (Fig. 1) and
human keratinocyte (Matsui et al., 2007) but was not
detected in canine normal skin and skin tumors. This
result suggests that Fat1 is not expressed in canine skin
or the expression level of Fat1 is quite low and that the
level of expression of Fat1 in skin differs between animal
spieces.

CONCLUSION

In this study, it is suggest from the present study
that Fat2 might relate in any way to canine skin tumors
such as cell proliferation. The further study of Fat2 in dog might be important for clarifying the mechanism of skin tumors and developing the treatment.

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REFERENCES


