Immunohistochemical Detection of p27 and p21 Proteins in Canine Hair Follicle and Epidermal Neoplasms

Makoto INOUE1, Haiyan WU1 and Satoshi UNE2

1)Departments of Veterinary Pathology, 2)Veterinary Hospital, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan

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ABSTRACT. Trichoblastomas, trichoepitheliomas, and squamous cell carcinomas in the skin of dogs were analysed by immunohistochemistry for the nuclear expression of p27, p21 and proliferating cell nuclear antigen (PCNA). High levels of p27 were present in trichoepitheliomas and trichoblastomas compared with squamous cell carcinomas. Detectable p21 was found in trichoepitheliomas and squamous cell carcinomas, but trichoblastomas had low level of p21 nuclear reactivity. Low levels of PCNA were detected in trichoepitheliomas and trichoblastomas compared with squamous cell carcinomas. The results suggested that nuclear p27 acts as a cyclin-dependent kinase (CDK) inhibitor in trichoepitheliomas and trichoblastomas. Nuclear p21 expression is involved in the induction of epithelial differentiation and seems to be unrelated to CDK inhibition.

KEY WORDS: canine cutaneous tumors, differentiation, p21, p27.

MATERIALS AND METHODS

Examined samples were selected from excisional biopsy specimens of skin tumors collected during a 3.5-year period (August 2000-February 2004) at Yamaguchi University. The samples consisted of formalin-fixed, paraffin wax-embedded tissue from 35 tumors; 14 trichoblastomas, 14 trichoepitheliomas, and seven squamous cell carcinomas. Sections from each sample were stained with hematoxylin and eosin, and histological diagnosis was based on the classification described by Goldschmidt and others [10].

Serial 5 µm sections were mounted on amino-silane-coated slides. The sections were dewaxed with xylene, rehydrated through graded ethanol, and microwave-treated in 10 mM citrate buffer, pH 6.0 for 6 min. All the following steps were conducted at 25°C. A biotin-streptavidin immunoperoxidase method was used. Endogenous peroxidase and avidin/biotin were quenched by immersion in 3% hydrogen peroxidase and endogenous avidin/biotin blocking solution (Nichirei Co., Ltd., Tokyo, Japan), respectively. The sections were blocked for 10 min in normal goat and rabbit sera (1:10 solution). The rabbit anti-human p27 and anti-human p21 antibodies, 200 µg/ml, 1:75 dilution (Santa Cruz Biotechnology, Santa Cruz, U.S.A.), and mouse monoclonal antibody for rat proliferating cell nuclear antigen (PCNA), 1:75 dilution (Novocastra Laboratories Ltd., Newcastle, UK) were applied to the tissues for 30 and 60 min, respectively. Biotinylated goat anti-rabbit IgG and rabbit anti-mouse IgG antibodies, 1:500 dilution (Nichirei Co., Ltd.) were used as secondary antibody for 20 min and peroxidase-conjugated streptavidin (Nichirei Co., Ltd.) was subsequently applied to the tissues. As the substrate chromogen, 3-amino-9-ethylcarbazol was used and the sections were then counterstained with hematoxylin.

Non-immune rabbit and mouse sera, as sources of irrelevant...
RESULTS

The results of the immunohistochemical p27, p21 and PCNA nuclear reactivity for each of the antibodies are summarized in Table 1. The percentage of p27-, p21- or PCNA-reactive tumor cells in each of cases is described.

In the epidermis of dogs, approximately 85% of normal keratinocytes in the malpighian and granular layers showed p27 reactivity, and basal cells showed occasionally (data not shown). In contrast, scattered p21 was seen in 35% of the basal compartments (data not shown). In the hair follicles, 75% of normal root-sheath and cortex cells were positive for p27, and 23% of the cells were positive for p21, but pilous matrix cells did not have detectable reactivity for the proteins (data not shown).

Each of the 14 trichoblastomas showed marked p27 (ranged from 56 to 83%; Fig. 1a). Only three of the tumors showed mild p21 (2, 3, 4%) and eleven showed none (Fig. 1b). In contrast to low level of p21 nuclear reactivity, each of the trichoblastomas had detectable p21 cytoplasmic reactivity (Fig. 1b). The cytoplasmic labelling was seen in many neoplastic cells through the whole area of the cellular masses.

Twelve of the 14 trichoepitheliomas showed marked p27 (ranged from 51 to 90%; Fig. 1c), and two showed moderate p27 (44, 47%). The p27 was detectable in most keratinized cells, and the percentage of p27-positive basaloid cells ranged from 65 to 90%. Eleven of the tumors showed moderate p21 (ranged from 13 to 49%; Fig. 1d), and three showed mild p21 (5, 7, 9%). The p21 was mostly seen in squamous cells, but not in basaloid and keratinized cells. A recent study has shown that canine squamous cell carcinomas showed moderate PCNA (44%) and six showed marked PCNA (57, 60, 64, 66, 79, 81%). In trichoepitheliomas and squamous cell carcinomas, PCNA reactivity was detected in squamous and basaloid cells, but not in keratinized cells. Low levels of PCNA reactivity were detected in trichoepitheliomas and trichoblastomas compared with squamous cell carcinomas, but no significant difference with PCNA was detected between the benign tumors.

DISCUSSION

Higher level of p27 nuclear expression and lower PCNA reactivity were present in trichoepitheliomas and trichoblastomas compared with squamous cell carcinomas. However, slightly decreased p27 expression was detected in the benign tumors compared with normal root-sheath and cortex cells. A recent study has shown that canine squamous cell carcinomas have significantly lower p27 positive index and higher proliferating index values than those of trichoepitheliomas and trichoblastomas, with the exception of pilomatrixomas [21]. Expression of p27 is preserved and may act as an inhibitory regulator of CDK activation in trichoepitheliomas and trichoblastomas.

Recently, it has been shown that p27 expression is induced in relation to retinoic acid-mediated growth arrest and differentiation of neuroblastoma cells [2, 17] and also differentiation of oligodendrocytes [8] and osteoblasts [7]. In the epidermis of dogs, high level of p27 nuclear expression was detectable in normal suprabasal keratinocytes, but not in basal cells. In the hair follicles, higher p27 expression

Table 1. Nuclear immunolabelling of canine hair follicle and epidermal neoplasms with antibodies to p27, p21, and PCNAa)

<table>
<thead>
<tr>
<th>Tumors</th>
<th>No. of dogs</th>
<th>p27 antibody</th>
<th>p21 antibody</th>
<th>PCNA antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Trichoblastomas</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trichoepitheliomas</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell carcinomas</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
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<td></td>
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a) − = absent; + = mild (1–10%); ++ = moderate (11–50%); +++ = marked (51–100%).
was detectable in root-sheath and cortex cells, but not in pilous matrix cells. In trichoepitheliomas and squamous cell carcinomas, p27 was seen in squamous and keratinized neoplastic cells, but was less common in basaloid cells. On the other hand, trichoblastomas consist of undifferentiated epithelial cells also had high level of p27 expression. The results suggest that p27 expression is detectable not only in differentiated cells but also in undifferentiated cells. It is considered that p27 nuclear expression is unrelated to epithelial differentiation of these tumor cells. Further studies should be carried out to understand the relationship between p27 and differentiation.

It has been reported that p21 expression correlates with normal differentiation in keratinocytes of the skin, but its expression is temporally present and then down-modulated at terminal differentiation [5]. In the present study, scattered p21 nuclear reactivity was detected in normal basal compartments in the epidermis and root-sheath and cortex cells in the hair follicles. Eleven of the 14 trichoepitheliomas and four of the seven squamous cell carcinomas showed moderate p21 nuclear reactivity, and eleven of the 14 trichoblastomas showed none. The p21 was mostly present in differentiating squamous cells, but not in basaloid and keratinized cells. Level of p21 nuclear expression was significantly higher in trichoepitheliomas than in trichoblastomas, although these tumors showed a similar p27 expression and proliferative activity. The results suggest that only p21 nuclear expression is involved in epithelial differentiation of the tumor cells and that the function of p21 seems to be unrelated to CDK inhibition within these tumors. In addition, higher p21 cytoplasmic reactivity was detected in trichoblastomas, but not in other tumors. Recent studies suggest that cytoplasmic p21 might act as regulators of CDK activation and apoptosis, and as transcriptional cofactors [4]. The functions of cytoplasmic p21 remain unclear in tumorigenesis.

In conclusion, higher p27 and lower PCNA nuclear expression were present in benign trichoepitheliomas and
trichoblastomas compared with squamous cell carcinomas. Nuclear p27 acts as a CDK inhibitor in trichoepitheliomas and trichoblastomas. By contrast, higher p21 nuclear expression was detectable in trichoepitheliomas and squamous cell carcinomas, but not in trichoblastomas. Nuclear expression of p21 is involved in the induction of epithelial differentiation and seems to be unrelated to CDK inhibition.

REFERENCES


