Topological Analysis of p21^{WAF1/CIP1} Expression in Esophageal
Squamous Dysplasia

Yasuhiro Shirakawa, Yoshio Naomoto, Masashi Kimura, Ryuichi Kawashima,
Tomoki Yamatsui, Takahiko Tamaki, Madoka Hamada, Minoru Haisa, and
Noriaki Tanaka
First Department of Surgery, Okayama University Medical School,
700-8558 Okayama Japan

ABSTRACT

In the normal stratified squamous epithelium of the esophagus, only the third to the fifth layers of cells express the cyclin-dependent kinase inhibitor p21^{WAF1/CIP1} (p21). Using immunohistochemical staining, we examined the topological distribution of cells expressing p21, p53, Ki67, and cytokeratin 10 (CK10), a differentiation marker of esophageal squamous cell carcinoma (SCC), in 25 superficial lesions and 72 dysplastic lesions of the esophagus. Image analysis of p21, p53, and Ki67 expression was also performed in 48 dysplastic lesions. In superficial SCCs, although Ki67- and p53-expressing cells were mainly distributed in the deep layers of tumors despite tumor differentiation, the distribution of p21 correlated with tumor differentiation. In dysplastic lesions, p53- and Ki67-coexpressing cells tended to locate in the same layers and expand in the lower layers of epithelium with the progression of dysplasia. p21-expressing cells shifted to the upper layers of the epithelium with the progression of dysplasia. However, this change was heterogeneous; in some lesions, p21-expressing cells were confined to the superficial layers of atypical cells (confined type), whereas in others, p21-overexpressing cells were scattered among atypical cells (scattered type). CK10 expression was observed in 25% of dysplastic lesions, and the frequency of CK10 expression was significantly higher in the scattered than in the confined type. Our results suggest that esophageal squamous dysplasia represents the earliest pathological process in esophageal squamous carcinogenesis. Our results also suggest that differentiation of esophageal SCC is determined at the stage of dysplasia, and that p21 plays a critical role in the differentiation process.

INTRODUCTION

Esophageal SCC has one of the worst prognoses among digestive carcinomas. At present, early detection is the best and only chance to improve the prognosis of esophageal SCC. Recent advances in diagnostic techniques have allowed a better detection of superficial esophageal SCCs, and EMR currently offers absolute curative therapy for some types of SCC superficial lesions. With regard to superficial SCCs, the importance of detection of dysplastic lesions that could be considered precancerous lesions has also been emphasized in recent years. Furthermore, various biomarkers of esophageal squamous dysplasia have been investigated, such as DNA content, Ki67, p53, and argyrophilic nucleolar organizer region (4). Results of these studies suggest that the nature of squamous dysplasia is analogous to that of intraepithelial SCC. In fact, some pathologists especially those in Japan, argue that this lesion is a low-grade carcinoma rather than a separate entity of esophageal squamous dysplasia. However, whether esophageal squamous dysplasia is a precancerous lesion remains to date a controversial issue, similar to the case of colonic adenoma in the adenoma-carcinoma sequence.

The cyclin-dependent kinase inhibitor p21^{WAF1/CIP1} was initially identified as a downstream effector of the cell cycle arrest function of p53. p21^{WAF1/CIP1} gene expression is directly up-regulated by wild-type but not mutant p53 at the transcriptional level (5, 6). However, p21^{WAF1/CIP1} gene is also regulated by p53-independent factors, including growth-promoting factors and differentiation-associated transcription factors (7–16). On the other hand, p21^{WAF1/CIP1} has been shown to be associated with senescence (17) and terminal differentiation (18–20). There is a striking compartmentalization of p21^{WAF1/CIP1}, expressing cells throughout the normal gastrointestinal tract epithelia. In the normal epithelium of the esophagus, only the third to fifth layers of cells express p21^{WAF1/CIP1}, whereas most cells in the second layer express Ki67, a well-known proliferating marker expressed during both the G1 and S phases of proliferation but not in quiescent cells (21). In recent years, expression of p21^{WAF1/CIP1} has been investigated in esophageal SCC as well as in tumors of other tissues such as the larynx, stomach, and head and neck (22–28). Several studies have shown a good correlation between the quantity and distribution of p21^{WAF1/CIP1} expression with proliferation and differentiation of SCC (23–25, 28). However, there has been only a few reports on the expression of p21^{WAF1/CIP1} in squamous dysplasia. Furthermore, the objective topological analysis of various biomarkers has never been reported in these lesions.

In the present study, using immunohistochemical staining...
and computer image analysis, we investigated the topological distribution of p21 WAF1/CIP1-, Ki67-, and p53-expressing cells in esophageal superficial SCC and dysplasia and determined the correlation between the two types of lesions in the course of esophageal squamous carcinogenesis. Furthermore, we performed immunohistochemical staining for CK10 in superficial esophageal SCC and dysplasia. CK10, an epidermis-related cytokeratin, is a marker of keratinizing squamous epithelium (29) that is not expressed in the normal esophageal epithelium. In esophageal SCC, the expression of CK10 correlates with the degree of keratinization, a feature of tumor differentiation (30). Our results showed CK10 expression not only in superficial SCC but also in dysplasia. We also investigated the correlation between p21 WAF1/CIP1 expression and the tendency for differentiation assessed by CK10 expression in dysplasia.

MATERIALS AND METHODS

Patients and Tissue Sampling. We examined 25 superficial SCCs, including 15 mucosal carcinomas and 10 submucosal carcinomas, obtained from previously untreated 23 patients. Patients included 21 men and 2 women with a mean age of 68.3 years (range, 44–73 years). We also examined 72 dysplastic lesions, including 20 mild, 22 moderate, and 30 severe dysplastic lesions, obtained from 53 patients. Patients were 47 men and 6 women with a mean age of 61.9 years (range, 38–76 years). Tissue samples were not obtained by biopsy because it has been our experience that such samples are often too small for image analysis. Instead, tissue samples were surgically or endoscopically resected at the First Department of Surgery, Okayama University Medical School, Okayama, Japan, during a period extending from 1992 to 1997.

The histological diagnosis of superficial SCC and squamous dysplasia was based on the WHO International Histological Classification of Tumors, 1990 (31). Superficial SCC represented mucosal and submucosal carcinomas, and the grade of tumor differentiation was assessed by the degree of keratinization. On the other hand, in mild dysplasia, atypical cells (which contain enlarged and hyperchromatic nuclei) were limited to the basal one-third of the thickness of the epithelium. In moderate dysplasia, this zone extends to two-thirds or less of the thickness of the epithelium, whereas in severe dysplasia, this zone extends to two-thirds or more. In the present study, intraepithelial SCCs were included in the severe dysplasia group because it was sometimes difficult to differentiate between intraepithelial SCC and severe dysplasia. Two experienced pathologists who were blinded to the clinical data and the results of other diagnostic tests determined the grade of dysplasia in all of the lesions.

Immunohistochemistry. p21 WAF1/CIP1, p53, Ki67, and CK10 staining was performed using formalin-fixed, paraffin-embedded serial sections. Tissue sections (3 µm thick) were mounted on silanized slides (DAKO Japan Co., Tokyo, Japan), were deparaffinized in xylene for 20 min, and were rehydrated in graded ethanol solutions. Endogenous peroxidase was blocked by incubating the sections in 3.0% H2O2 in methanol for 15 min. Antigen retrieval on paraffin sections was performed.
Expression of p21WAF1/CIP1 and Ki67 was considered positive if CK10 assessment was based on the cytoplasmic staining pattern. Ki67 was based on the nuclear staining pattern, whereas the subsequent application of a biotinylated antiprimary antibody and streptavidin-peroxidase (Histofine SAB PO kit; Nichirei, Tokyo), sections were counterstained with Mayer’s hematoxylin. As a negative control, some sections were subjected to normal serum blocking and omission of the primary antibody. In each lesion, the assessment of p21 WAF1/CIP1, p53, and Ki67 expressions in squamous dysplasia, digital image analysis was performed using NIH Image analysis program.3 For objective estimation of the topological distribution, we used the TI, calculated as follows: (a) using NIH image analysis software, immunoreactive cells were marked manually, and, at the same time, the coordinates (x, y) of each marked cell were plotted individually; (b) the top (luminal) and bottom (basal) side of the epithelium were marked manually, [the coordinates of the top marker were (x0, y0), and those of the bottom marker were (x, y)]; and (c) r = (y0 - y)/(y0 - y0), where r represented the relative value of the vertical position of immunoreactive cells in the epithelium. The mean and SD of r values of all of the immunoreactive cells was defined as the TI, and this index reflected the topology of the immunoreactive area in the epithelium (Fig. 1).

**Statistical Analysis.** Differences between groups were examined for statistical significance using the unpaired t test (Student, Welch) and the χ² test. A P less than 0.05 denoted the presence of a statistically significant difference.

### Table 1 Summary of p21WAF1/CIP1, CK10, p53 expression in superficial SCC

<table>
<thead>
<tr>
<th>Lesion no.</th>
<th>Age</th>
<th>Sex</th>
<th>Differentiation</th>
<th>Depth of invasion</th>
<th>Therapy</th>
<th>p21 expression</th>
<th>CK10 expression</th>
<th>p53 expression</th>
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<td>1</td>
<td>74</td>
<td>M</td>
<td>Well</td>
<td>m</td>
<td>EMR</td>
<td>Scattered</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>M</td>
<td>Well</td>
<td>m</td>
<td>Operation</td>
<td>Scattered</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
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<td>Well</td>
<td>m</td>
<td>Operation</td>
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<td>Positive</td>
</tr>
<tr>
<td>4</td>
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<td>F</td>
<td>Well</td>
<td>m</td>
<td>Operation</td>
<td>Scattered</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>M</td>
<td>Well</td>
<td>sm</td>
<td>Operation</td>
<td>Scattered</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>M</td>
<td>Well</td>
<td>sm</td>
<td>Operation</td>
<td>Scattered</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>M</td>
<td>Well</td>
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</tr>
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<td>Positive</td>
</tr>
<tr>
<td>9</td>
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<td>Scattered</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
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<td>Positive</td>
</tr>
<tr>
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<td>m</td>
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<td>m</td>
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<td>Positive</td>
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<tr>
<td>23</td>
<td>62</td>
<td>M</td>
<td>Poorly</td>
<td>sm</td>
<td>Operation</td>
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<td>69</td>
<td>M</td>
<td>Poorly</td>
<td>sm</td>
<td>Operation</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*a*  m, mucosal carcinoma; *sm*, submucosal carcinoma.

10% of the atypical cells examined. Furthermore, the percentage of immunoreactive cells in the lesions was semiquantitatively evaluated by counting at least 500 cells in the most representative area.

**Monoclonal Antibodies.** Clone EA10 for p21WAF1/CIP1, p53, and Ki67 was based on the nuclear staining pattern, whereas the CK10 assessment was based on the cytoplasmic staining pattern. Expression of p21WAF1/CIP1 and Ki67 was considered positive if it was equal or stronger than its expression in normal epithelium. Expression of p53 and CK10 was considered positive when it was equal or stronger than its expression in normal epithelium.

Immunoreactive area in the epithelium (Fig. 1). Defined as the TI, and this index reflected the topology of the immunoreactive area in the epithelium (Fig. 1).
RESULTS

Distribution of p21^WAF1/CIP1, p53, and Ki67 in Esophageal Superficial SCC. p53 was overexpressed in 23 (92%) of 25 esophageal superficial SCCs. In most lesions, the distribution of p53-expressing cells was similar to that of Ki67-expressing cells; they were mainly located in the deep layers of the tumor. This distribution was independent of the grade of tumor differentiation (Table 1; Figs. 2 and 3).

p21^WAF1/CIP1 immunoreactivity was detected in 13 (59%) of 25 of esophageal superficial SCCs. Expression of p21^WAF1/CIP1 correlated with the grade of differentiation. Expression of p21^WAF1/CIP1 was high in 8 (100%) of 8 well-differentiated and 4 (57%) of 7 moderately differentiated but was faint in 4 (40%) of 10 poorly differentiated superficial SCCs. The latter pattern of expression was also limited to the top layer of the tumor. In differentiated SCCs, p21^WAF1/CIP1-expressing cells were mostly scattered in the upper and middle layers of the tumor and were considerably

Fig. 2 Expression of p21 (a), Ki67 (b), p53 (c), and CK10 (d) in well-differentiated esophageal superficial SCCs. Ki67- and p53-expressing cells are located mainly in the deep layers of the tumor, and the distribution of p21^WAF1/CIP1-expressing cells shows overexpression and mixing of these cells with Ki67 expressing cells. The presence of CK10 expression is notable.

Fig. 3 Expression of p21^WAF1/CIP1 (a), Ki67 (b), p53 (c), and CK10 (d) in poorly differentiated esophageal superficial SCC. Similar to well-differentiated lesions, Ki67- and p53-expressing cells are mainly located in the deep layers of the tumor. However, faint p21^WAF1/CIP1-expression was found only in the top layers of the tumor. There is a lack of CK10 expression.
intermingled with those of Ki67-expressing cells. However, p21 WAF1/CIP1 and Ki67 expression was mutually exclusive at the cellular level. In keratinized areas, peripheral cell layers were positive for Ki67, whereas adjacent internal layers were positive for p21WAF1/CIP1 (Table 1; Figs. 2 and 3).

**Table 2** Summary of p21 WAF1/CIP1, Ki67, p53, and CK10 expression in dysplasia

<table>
<thead>
<tr>
<th>Lesion no.</th>
<th>Age/Sex</th>
<th>Grade of dysplasia</th>
<th>TI of p21 - TI of Ki67</th>
<th>p53 expression</th>
<th>CK10 expression</th>
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<tr>
<td>1</td>
<td>40/M</td>
<td>Mild</td>
<td>ns</td>
<td>Positive (ns)</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>75/M</td>
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<td>Positive (ns)</td>
<td>Negative</td>
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<tr>
<td>3</td>
<td>64/M</td>
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<td>P &lt; 0.05</td>
<td>Positive (ns)</td>
<td>Negative</td>
</tr>
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<td>4</td>
<td>72 /F</td>
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<td>ns</td>
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<tr>
<td>5</td>
<td>53/M</td>
<td>Mild</td>
<td>P &lt; 0.05</td>
<td>Positive (ns)</td>
<td>Negative</td>
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<tr>
<td>6</td>
<td>74/M</td>
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<td>P &lt; 0.01</td>
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<td>Negative</td>
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<tr>
<td>7</td>
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<td>Mild</td>
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<td>Negative</td>
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<tr>
<td>8</td>
<td>67/M</td>
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<td>39</td>
<td>60/M</td>
<td>Severe</td>
<td>ns</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>40</td>
<td>71/M</td>
<td>Severe</td>
<td>P &lt; 0.01</td>
<td>Positive (ns)</td>
<td>Negative</td>
</tr>
<tr>
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<td>67/M</td>
<td>Severe</td>
<td>P &lt; 0.01</td>
<td>Positive (ns)</td>
<td>Negative</td>
</tr>
<tr>
<td>42</td>
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<td>P &lt; 0.01</td>
<td>Positive (ns)</td>
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</tr>
<tr>
<td>43</td>
<td>68/M</td>
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<td>P &lt; 0.01</td>
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<td>Negative</td>
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<tr>
<td>44</td>
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<td>Negative</td>
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<tr>
<td>46</td>
<td>72 /F</td>
<td>Severe</td>
<td>P &lt; 0.01</td>
<td>Positive (ns)</td>
<td>Positive</td>
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<tr>
<td>47</td>
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<td>Negative</td>
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<tr>
<td>48</td>
<td>72 /F</td>
<td>Severe</td>
<td>P &lt; 0.01</td>
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<td>Negative</td>
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</table>

*ns, not significant.

Distribution of p21 WAF1/CIP1, p53, and Ki67 in Esophageal Squamous Dysplasia. The percentages of esophageal squamous dysplastic lesions that were positive for p21 WAF1/CIP1 and p53 were 92% (66 of 72) and 78% (56 of 72), respectively. We were able to use 48 (67%) of 72 lesions for image analyses of various biomarkers in the following studies. These consisted of 12 mild, 16 moderate, and 20 severe dysplastic lesions (Table 2).

No p53-expressing cells were detected in eight dysplasias, and there was no significant difference between the TI of p53 and that of Ki67 in 82% (33 of 40) of p53-positive lesions. With the progression of dysplasia, the distribution of Ki67- and p53-expressing cells expanded in the lower layers of the epithelium in many lesions (Table 2; Fig. 4).

Overall, the distribution of p21 WAF1/CIP1-expressing cells shifted to the upper layers of the epithelium with the progression of dysplasia. Furthermore, there were significant differences in...
the mean r-values of p21\textsuperscript{WAF1/CIP1}-expressing cells among each grade (P < 0.01). However, this change was heterogeneous. In some lesions, p21\textsuperscript{WAF1/CIP1}-expressing cells were found in a few lines among the superficial layers of atypical cells, whereas in others, cells overexpressing p21\textsuperscript{WAF1/CIP1} were scattered among atypical cells. In the latter group, p21\textsuperscript{WAF1/CIP1}-expressing cells were mixed with those of Ki67-expressing cells. There were 22 dysplastic lesions (46%) in which no significant difference was detected in TI between p21\textsuperscript{WAF1/CIP1}-expressing cells and Ki67-expressing cells. However, similar to superficial SCC, p21\textsuperscript{WAF1/CIP1} and Ki67 expression was mutually exclusive to the cellular level in dysplastic lesions (Table 2; Figs. 5 and 6).

**Fig. 5** TI of p21\textsuperscript{WAF1/CIP1}-expressing cells in esophageal squamous dysplasia. Overall, the topological distribution of p21\textsuperscript{WAF1/CIP1}-expressing cells shifted significantly to the upper layers of the epithelium (P < 0.01).

**Fig. 4** TI of Ki67- and p53-expressing cells in esophageal squamous dysplasia. No p53-expressing cells were detected in 8 dysplastic lesions, and there was no significant difference between the TI of p53-expressing cells and the TI of Ki67-expressing cells in 33 dysplastic lesions. With the progression of dysplasia, the topological distribution of Ki67- and p53-expressing cells overlapped with each other and expanded in the lower layer of the epithelium in many lesions.

**Fig. 5** TI of p21\textsuperscript{WAF1/CIP1}-expressing cells in esophageal squamous dysplasia. Overall, the topological distribution of p21\textsuperscript{WAF1/CIP1}-expressing cells shifted significantly to the upper layers of the epithelium (P < 0.01).

**Fig. 6** TI of Ki67- and p53-expressing cells in esophageal squamous dysplasia. No p53-expressing cells were detected in 8 dysplastic lesions, and there was no significant difference between the TI of p53-expressing cells and the TI of Ki67-expressing cells in 33 dysplastic lesions. With the progression of dysplasia, the topological distribution of Ki67- and p53-expressing cells overlapped with each other and expanded in the lower layer of the epithelium in many lesions.
Relationship between Distribution of \( p21^{\text{WAF1/CIP1}} \)-expressing Cells and Proliferation and Differentiation of Esophageal Squamous Dysplasia. The relationship among heterogeneous \( p21^{\text{WAF1/CIP1}} \) expression, differentiation, and proliferation was investigated in severe dysplastic lesions (\( n = 20 \)). As stated earlier, severe dysplastic lesions were classified in the present study into two types: confined type and scattered type. In the confined type (\( n = 10 \)), \( p21^{\text{WAF1/CIP1}} \)-expressing cells were confined to the upper one-half of layers of the epithelium, whereas in the scattered type (\( n = 10 \)), \( p21^{\text{WAF1/CIP1}} \)-expressing cells were found beyond the upper one-half of layers. To investigate the tendency of these two different types of dysplasias for differentiation, serial sections were immunostained for CK10. There was a significant differ-

![Fig. 6](image-url)  
**Fig. 6** \( p21^{\text{WAF1/CIP1}} \) expression and TI values in normal esophageal epithelium (a), moderate dysplasia (b, d) and severe dysplasia (c, e). Overall, \( p21^{\text{WAF1/CIP1}} \)-expressing cells shifted to the upper layers of the epithelium. However, \( p21^{\text{WAF1/CIP1}} \) expression in dysplasia was heterogeneous. In the present study, severe dysplastic lesions were classified as confined type (c) and scattered type (e) by estimating the topological distribution of \( p21^{\text{WAF1/CIP1}} \)-expressing cells.

![Fig. 7](image-url)  
**Fig. 7** CK10 expression in severe dysplastic esophageal lesions of scattered type.
Table 3  Relationship between type of p21WAF1/CIP1 expression and CK10 expression in severe dysplastic lesions

<table>
<thead>
<tr>
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<th>CK10 expression</th>
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</thead>
<tbody>
<tr>
<td>Confined type (n = 10)</td>
<td>Positive 1 (10.0%)  Negative 9 (90.0%)</td>
</tr>
<tr>
<td>Scattered type (n = 10)</td>
<td>Positive 6 (60.0%)  Negative 4 (40.0%)</td>
</tr>
</tbody>
</table>

* P < 0.05 with statistic significant.

ence in CK10 expression between scattered type and confined type (P < 0.05). CK10-expressing cells were detected in 60% (6 of 10) of scattered-type lesions but in only 1 (10%) of 10 lesions in the confined-type lesions (Table 3; Fig. 7). Furthermore, the topological distribution of CK10 seemed to overlap with that of p21WAF1/CIP1 in dysplastic lesions (Figs. 6e and 7). To investigate the proliferative activity, we determined the percentage of Ki67-expressing cells in these lesions. There was no significant difference in the percentage of Ki67 expressing cells between confined and scattered lesions (Fig. 8).

DISCUSSION

In the present study, we focused on the topological distribution of p21WAF1/CIP1, Ki67-, and p53-expressing cells in esophageal squamous dysplasia and superficial SCC to characterize esophageal squamous carcinogenesis, proliferation, and differentiation. In normal squamous epithelium of the esophagus and other tissues, p21WAF1/CIP1-expressing cells are located above the proliferative compartment (which express Ki67) in parabasal layers (21), and cells with only faint expression of p53 are detected on rare occasions (25). In the present study, the distribution of Ki67 in many dysplastic lesions overlapped with that of p53-expressing cells but expanded in the lower layers of epithelium with the progression of dysplasia. On the other hand, Ki67- and p53-coexpressing cells were mainly located in the deep layers of many superficial SCCs. Overall, in dysplastic lesions, the distribution of p21WAF1/CIP1-expressing cells shifted to the upper layers of the epithelium with the progression of the grade of dysplasia. In superficial SCCs, p21WAF1/CIP1-expressing cells were mainly located in the upper layer of the tumor. On the basis of these findings, it seems that esophageal SCCs arise in the parabasal layer of the epithelium, and dysplasia is the earliest pathological process that grows upward toward the luminal surface. Our results also showed that p53 gene mutation correlated with esophageal squamous carcinogenesis and tumor progression in the early stages in many lesions.

Heterogeneous expression of p21WAF1/CIP1 was observed not only in SCC but also in dysplasia. In esophageal superficial SCCs, the pattern and topological distribution of p21WAF1/CIP1 expression correlated with the grade of differentiation. In recent years, a similar correlation between p21WAF1/CIP1 expression and tumor differentiation has been reported in esophageal SCCs (23, 25) as well as in head and neck SCCs (24, 28), cutaneous SCCs, non-small cell lung carcinomas (32), and colon adenocarcinomas (33). However, to our knowledge, there are no reports on the heterogeneous expression of p21WAF1/CIP1 in squamous dysplasia. Yang et al. (25) reported that p21WAF1/CIP1-expressing cells were found surrounding the top of the hyperplastic region. In other studies, p21WAF1/CIP1-expressing cells were detected throughout the whole thickness of the epithelium in severe dysplasia (24, 28). Our results showed that in some dysplastic lesions (confined type), p21WAF1/CIP1-expressing cells were present in a few lines in the superficial layers of atypical cells similar to p21WAF1/CIP1-positive, poorly differentiated-type superficial SCCs. In dysplastic lesions of the scattered type, p21WAF1/CIP1-overexpressing cells were scattered among atypical cells similar to differentiated-type superficial SCCs. Furthermore, CK10, a marker of differentiation of SCC, was detected in dysplastic lesions, and the pattern of p21WAF1/CIP1 expression correlated with CK10 expression in a manner similar to that observed in SCCs. Taken together, these findings suggest that the tendency for differentiation of SCC is initiated at the early stages of carcinogenesis, and p21WAF1/CIP1 expression seems to be involved in differentiation.

Overexpression of p21WAF1/CIP1 is associated with suppression of tumor growth in experimental models (18). However, p21WAF1/CIP1 expression might not overcome the progression of esophageal squamous dysplasia and SCC. In dysplasia, the heterogeneity of p21WAF1/CIP1 expression did not correlate with the proliferative activity assessed by the percentage of Ki67-expressing cells, and similar phenomenon has been described in SCC of different tissues using several proliferation markers (28, 34).

Several studies have demonstrated p53-independent expression of p21WAF1/CIP1 in different normal and malignant tissues (21, 24, 34–36). However, in the present study, it was not clear whether p21WAF1/CIP1 expression in squamous dysplasia...
sia was independent of p53 gene status. Although p53-positive severe dysplastic lesions showed both confined-type and scattered-type p21WAF1/CIP1 expression, all of the p53 negative severe dysplastic lesions showed scattered-type p21WAF1/CIP1 expression. We, therefore, speculate that the pattern of p21WAF1/CIP1 expression in esophageal squamous dysplasia tends to depend on p53 gene status and function. However, it could also be debated that p53 overexpression is not always synonymous with p53 gene mutation. Overestimation of p53 gene mutation by immunohistochemistry could sometimes be related to certain mechanisms including inactivation of the enzymatic pathway responsible for p53 degradation (37) or inactivation of wild-type p53 through binding with another protein that prolongs its half-life (38). False-negative cases represent about 10% of all of the p53 gene mutations and preferentially occur in certain types of p53 gene mutation (39, 40). Additional molecular biological studies are, thus, necessary to clarify the mechanism of p21WAF1/CIP1 expression in differentiation of esophageal dysplasia and to address whether p21WAF1/CIP1 expression alone could explain differentiation of esophageal squamous dysplasia and esophageal SCC.

In conclusion, we suggest that esophageal squamous dysplasia is the earliest pathological process in esophageal squamous carcinomaogenesis. The degree of differentiation of esophageal SCC may be determined at the stage of dysplasia and is correlated with p21WAF1/CIP1 expression. It is, therefore, essential to follow up these early lesions at appropriate intervals and to resect at an appropriate stage. Furthermore, p21WAF1/CIP1 expression may be a useful indicator for treating esophageal squamous dysplasia.

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Topological Analysis of p21$^{WAF1/CIP1}$ Expression in Esophageal Squamous Dysplasia

Yasuhiro Shirakawa, Yoshio Naomoto, Masashi Kimura, et al.


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