The Individual Cell Properties of Oral Squamous Cell Carcinoma and p53 Tumor Suppressor Gene Mutation

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Abstract: There is no consensus on the relationship between variations in TP53 mutations and tumor properties in oral squamous cell carcinoma (OSCC). To further the basic research required to eventually develop individualized (order-made) treatments and prognoses for OSCC, we established six human OSCC lines from patients within our department. Together with another nine cell lines derived from donations by other organizations, we determined the TP53 mutation and single nucleotide polymorphism (SNP) of codon 72 in a total of 15 cell lines, and examined in vitro cell invasion activity and anti-cancer drug sensitivity. The missense mutation at codon 248 was most abundant, and was noted in four cell lines, but other diverse mutation variations were also revealed. The cells which expressed the mutated p53 protein (the p53(+) group) showed slightly higher invasion activity than did the p53(−) group. In p53(+) group, the 72R of SNP (72P/R) was higher than the 72P in invasion activity, although the difference was not significant. Surprisingly, an anti-cancer drug sensitivity test with four different types of drugs showed that the p53(−) group was more resistant in other than CDDP, and that 72R was more sensitive than 72P in the p53(+) group. To clarify the characteristics of the R248Q mutation, which is the most abundant missense mutation, the gene was introduced with an expression plasmid vector into a TP53 null Saos-2 cell. The transformant of R248Q mutation gained higher activity of invasion, while its anti-cancer drug sensitivity also increased. Our findings suggest that it may be possible to estimate oral cancer cell characteristics and the malignancy level based on differences in the TP53 mutation.

Key words: Oral squamous cell carcinoma, p53 Tumor suppressor gene, Cell invasion activity, Anti-cancer drug susceptibility
loss of various functions mediated by the transcription of p53 target genes, such as stopping of the cell cycle, apoptosis, inhibition of capillary vascularization, and DNA restoration. Consequently, the cells transform into malignancies and are involved in immortal conversion, derepression of cell multiplication, and the acquisition of DNA chromosome instability. This is also related to other malignant characteristics of cancer cells, such as resistance to anti-cancer drugs and radiation, cell infiltration conversion, and increased metastatic activity.

However, the variations of the missense mutation of TP53 are numerous and, by 2004, there were about 1000 kinds of amino acid substitution reported in the literature. Therefore, it has been suggested that the variety of TP53 mutations reflects the variety of acquired individual cancer properties. In non-small cell carcinoma of lung cancer (NSCLC) and breast cancer, TP53 mutations have been reported to be negative prognostic factors of the 5 year survival rate. In colon cancer and bladder cancer, by contrast, only about half of the reports have related the TP53 mutation with the prognosis. For oral cancer, there are too few reports to allow an adequate systematic review or meta-analysis. Those reports that have dealt with the relationship between the TP53 mutation and drug sensitivity have been contradictory, with some showing positive correlation and others a negative correlation. In addition, not only the mutation of TP53 but also the SNP (single nucleotide polymorphism) of the 72nd codon with an amino acid substitution has been reported. The 72P type of CCC codes the proline and the 72R type of CGC codes the arginine, and the allele frequency among Japanese patients has been reported to be 1.58. The same group reported that the mutated TP53 with 72R did not have a dominant negative effect and that, in lung cancer, the 72P was associated with a worse prognosis than was the 72R, which was the inverse of the finding for breast cancer. In terms of their anti-cancer drug sensitivity, the wild type p53 showed more sensitivity than did the mutated p53. The same group reported that the anti-cancer drug sensitivity increased when the wild type p53 was introduced into cells possessing the mutated p53. However, the clinical findings regarding the relationship between the p53 mutation and anti-cancer drug sensitivity suggest a more complex dynamic which involves not simply the type of TP53 mutation or SNP of codon 72, but also other factors such as the expression of the p53 family member, p63 and p73, the type of anti-cancer drug, and even the tissue specificity or tumor type. While it appears that the TP53 mutation plays an important function in normal cells, is universally mutated in many cancer cells, and may be utilized as a diagnostic and prognostic factor in some cancers, there is as yet insufficient data to justify more specific conclusions given the contradictory findings and confounding variables involved. In the present study, we focused on culturing cells from OSCC cases to examine the relationship between cell characteristics and the TP53 mutation or polymorphism (SNP).

Materials and methods

This study was conducted with the approval of the ethical review board of Tsurumi University. Informed consent was obtained from all of the patients prior to this study.

1. Case, established cell line, transfection, and cell culture

A part of the tissues from the biopsy and excision operation of oral cancer patients who visited the Oral and Maxillo-facial Surgery Department from 2002 to 2004 was cultured in vitro, and the human oral cancer cell line was established as follows.

The tissue was cut finely and culture was started in a flask with Dulbecco’s Modified Eagle Medium (DMEM) “Nissui” (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), 0.2% NaHCO3, L-Glutamine (Nissui Pharmaceutical) supplemented with 20% fetal bovine serum (FBS) under a 37°C, 5% CO2 atmosphere condition. The culture was continued while changing the nutrient medium until the out-grown cell from the fragment of tissues became confluent. Consequently, the cells were repeatedly transferred to a new laboratory dish several times. Established cell lines supplied from other organizations, Japanese Cell Resources Bank (JCRB) or American Type Culture Collection (ATCC), SCC cell lines of oral cancer origin, KB, HSC-3, HSC-4, SAS, SCC25, Ca9-22, HOC313, HO-1-u-1, HO-1-N-1, a line of osteogenic sarcoma origin, Saos-2, and normal fibroblast
cell line, TIG-7, were also cultured with it, with the DMEM supplemented with 10% FBS.

The plasmid constructs inserted the mutated TP53 gene under the CMV promoter; pCMVneo248Q and pCMVneo273H (kindly donated by Professor N. Tsuchida, Tokyo Medical and Dental University) were used for the mutated TP53 expression vectors. Two μg of the purified plasmids was transfected into 3 × 10^5 cells of TP53 null cell line Saos-2 by the DNA-calcium phosphate coprecipitation method. The transformants were selected with neomycin analog G418 (400 μg/ml), designated as Saos248Q and Saos273H or Saos neo as control which was transfected by only a vector, and were cultured with the DMEM supplemented with 10% FBS.

2. cDNA synthesis, amplification, and sequence analysis

From each cell line, total RNA was extracted by using the High pure RNA Isolation Kit (Roche Diagnostics Co., Indianapolis, USA). The cDNA was synthesized by reverse transcription reaction with SuperScript II Rnase H- Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). The full length of the TP53 coding region was amplified in each cDNA with p53 specific primers: p5312F (5'-acggtgacacgcttccctggattgg-3'), p5312R (5'-gctgctcagatagcgatg-3') and PfuTurbo HotStart DNA polymerase with reaction buffer (Stratagene, La Jolla, CA, USA). The reaction conditions were as follows. After the first denaturation at 95°C for 3 min, 35 cycles reaction at 94°C, 1 min., 60°C, 1 min. and 72°C, 2 min. were performed, and the final extension reaction was 72°C, 7 min. After confirming the amplification by agarose gel electrophoresis, the PCR product was purified with GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences Corp. NJ and USA), and the sequence reaction was carried out with primers p5312F, p5312R, p53FAS (5'-acaagcacaatttcttcac-3') and p53RS (5'-gtgctcagatagcttggt-3'), by using ABI Prism BigDye Terminator V3.1 cycle sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the instructions of the manufacturer. The base sequence of each reaction product was analyzed with ABI Prism 3730X DNA analyzer (Applied Biosystems).

3. Quantitative real-time PCR

The equipment used was LightCycler (Roche) and LightCycler software Version 3.5 (Roche). The cDNA prepared as described above was used as a template. As for analysis of TP53 gene and GAPDH gene expression level, the Quanti Tect Probe PCR Kit (Quiagen, Hilden and Germany) and LightCycler-FastStart DNA master SYBR Green I kit were used with 1 mM of MgCl2. The reaction conditions were as follows. After the first denaturation at 95°C for 10 min., 45 cycles of reaction at 95°C, 0 second, 60°C, 5 sec., and 72°C, 15 sec were performed. The control plasmid DNA pProSp53 (supplied from the JCRB gene bank) was measured at the same time as a standard. The amounts of expression of the GAPDH gene which was the house-keeping gene as an internal standard in each cell line, were examined and compared.

4. p53 protein determination by ELISA

The cultured cell line was extracted by using Cell Extraction Buffer (Biosource International Inc., USA and Camarillo, CA, USA) and used as a sample. The p53 protein amounts in each line were determined with the ELISA kit of DuoSet IC Human Total p53 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions and the measurement of the absorbance with 415 nm (reference wavelength 550 nm) in an Ultramark microplate reader (BIO-RAD, Richmond, CA, USA). The quantity of p53 protein was divided by the total protein amount of each cell to compare the p53 level with each other. The total protein was measured with Protein Assay Kit I (BIO-RAD) based on the Bradford method, according to the manufacturer’s instructions.

5. In vitro cell invasion assay

To evaluate each cell invasion activity, the absorbance of extracted chromogen which stained migrated cells through the membrane with extracellular matrix was measured with the Cell Invasion Assay Kit (Chemicon International Inc., Temecula, CA, USA)\(^{20, 21}\). A statistical analysis of measurements was performed and p < 0.05 was judged to be a significant difference in the Mann-Whitney U test by using the computer program, Stat View-J5.0.

6. Anti-cancer drugs susceptibility test

The anti-cancer drugs susceptibility was measured by MTT (3-[4, 5-dimethylthiazol]-2,5diphenyltetrazolium
bromide) assay, essentially as described previously\textsuperscript{24}. In short, each cell line was adjusted to the density of 1 $\times$ 10^4 cells/ml, divided into 100 $\mu$l per well of a 96-well plate (Falcon Becton Dickinson and Co., Franklin Lakes, NJ., USA), and cultured for two hours with the DMEM medium supplemented with 10% FBS. The sequential two-fold dilution of the drugs, vincristine sulfate (VCR) (Japanese Eli Lili Ltd.), peplomycin sulfate (PEP) (Nippon Kayaku Ltd.), cisplatin (CDDP) (Nippon Kayaku Ltd.), Doxorubicin hydrochloride (DOX) (Sigma-Aldrich, Tokyo, Japan) of 100 $\mu$l was added to each well and cultured in the presence of the drug for two days. To the culture, 50 $\mu$l of MTT (Thiazolyl Blue Tetrazolium Bromide) (Sigma) was added and further cultivated for four hours. After removing the culture supernatant, 150 $\mu$l of DMSO was added into the wells to solve the formazan metabolized from MTT and the absorbency of 550 nm (reference wavelength 630 nm) was measured with an Ultramark microplate reader (BIO-RAD). The experiment in each cell line was repeated two to four times independently, and the mean value of 50% inhibitory concentration (IC\textsubscript{50}) was calculated. A statistical analysis of data was performed and p < 0.05 was judged to be a significant difference in the Mann-Whitney U test by using the computer program, Stat View-J5.0.

Results

1. The cases and establishing the cell line

Six cell lines from 81 cases were established in vitro. Table 1 shows the characteristics of six cases. Four patients were males and two were females; their average age was 58 years old, with a range of 38 to 88 years. The original cancer region was the tongue, in four cases, the buccal mucosa in one case, and the oral floor in one case. Three patients were positive for lymph node metastasis. The periods from the start of culture to the first passage of TOS-1TY was 5 months, TOS-2FY was 3 months, TOS-3KS was 4 months, TOS-4CO was 3.5 months, TOS-5MI was 3 months, and of TOS-6AS was 3.5 months. Passage continued in each case for about 12 months.

2. Mutation and SNP analysis of TP53 gene in established cell lines

cDNA obtained from the six novel cell lines we established and the nine cell lines obtained from other organizations was analyzed for the TP53 gene mutation and SNP by sequencing.

(1) Mutation analysis

Mutation of the p53 gene was recognized in most of the cell lines (Fig. 1 & Table 2).

Most of the mutations were of the missense type. The only cell line without mutations was KB. HSC-4 had a missense mutation on codon 248 changing the arginine code to a glutamine code. HO-1-N-1 and TOS-3KS showed the same mutation variation code as HSC-4 (Fig. 1-a), and in Ca9-22 the missense mutation on codon 248 changed arginine code to tryptophan code. In TOS-2FY, 3 bps were deleted which coded phenylalanine on codon 113. In TOS-5MI, glycine code was turned into an alanine code by a 1 bp deletion of codon 105 and consequently, a frame shifted. In TOS-4CO, the serine of codon 94 turned into OPA (Stop codon) (Fig. 1-b). In TOS-1TY, the rearrangement had appeared between codon 281 and the Stop codon. TOS-6AS showed re-arrangement from codon 59 to codon 184. In HOC313, codon 285 changed the code to lysine from glutamic acid. In HO-1-u-1, there was one base insertion between codon 292-293 to change codon 293 of glycine to a tryptophan code, and the following frame

<table>
<thead>
<tr>
<th>Cases</th>
<th>Sex</th>
<th>Age</th>
<th>Primary site</th>
<th>TNM Stage</th>
<th>Chemotherapy before culture</th>
<th>Operation</th>
<th>Present status</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOS-1TY</td>
<td>male</td>
<td>74</td>
<td>tongue</td>
<td>T1N0M0</td>
<td>Peplomycin $\Sigma$ 30mg</td>
<td>+</td>
<td>Alive</td>
</tr>
<tr>
<td>TOS-2FY</td>
<td>male</td>
<td>88</td>
<td>buccal mucosa</td>
<td>T3N1M0</td>
<td>—</td>
<td>—</td>
<td>Dead</td>
</tr>
<tr>
<td>TOS-3KS</td>
<td>female</td>
<td>38</td>
<td>oral floor</td>
<td>T3N1M0</td>
<td>—</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td>TOS-4CO</td>
<td>female</td>
<td>62</td>
<td>tongue</td>
<td>T2N2CM0</td>
<td>Peplomycin $\Sigma$ 20mg</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td>TOS-5MI</td>
<td>male</td>
<td>44</td>
<td>tongue</td>
<td>T1N0M0</td>
<td>Peplomycin $\Sigma$ 15mg</td>
<td>+</td>
<td>Alive</td>
</tr>
<tr>
<td>TOS-6AS</td>
<td>male</td>
<td>41</td>
<td>tongue</td>
<td>T2N0M0</td>
<td>—</td>
<td>+</td>
<td>Alive</td>
</tr>
</tbody>
</table>

Histopathological diagnosis: all cases SCC
Fig. 1  Data of direct DNA sequencing of the representative cases in Table 2. 
(a) Missense of codon 248 revealed by sequence analysis for amplified p53 cDNA obtained from TOS-3 KS cells and TP53 wild type cells (KB).
(b) Nonsense of codon 94 revealed by sequence analysis for amplified p53 cDNA obtained from TOS-4CO cells and TP53 wild type cells (KB).

Table 2  Analysis of TP53 mutation and SNP of codon 72

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>exon</th>
<th>codon</th>
<th>Base (amino acid) changing</th>
<th>mutation</th>
<th>Codon72 SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB</td>
<td>N.D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSC-3</td>
<td>8</td>
<td>305-360</td>
<td>TAAG insertion (frameshift)</td>
<td>4bp insertion</td>
<td>CCC(P)</td>
</tr>
<tr>
<td>HSC-4</td>
<td>7</td>
<td>248</td>
<td>CGG (R) → CAG (Q)</td>
<td>missense</td>
<td>CGC(R)</td>
</tr>
<tr>
<td>SAS</td>
<td>10</td>
<td>336</td>
<td>GAG (E) → TAG (AMB:STOP)</td>
<td>nonsense</td>
<td>CGC(R)</td>
</tr>
<tr>
<td>SCC25</td>
<td>4</td>
<td>106</td>
<td>AGC (S) → ATC (I)</td>
<td>missense</td>
<td>CGC(R)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>209</td>
<td>GA deletion (frameshift)</td>
<td>2bp deletion</td>
<td></td>
</tr>
<tr>
<td>Ca9-22</td>
<td>7</td>
<td>248</td>
<td>CGG (R) → TGG (W)</td>
<td>missense</td>
<td>CCC(P)</td>
</tr>
<tr>
<td>HOC313</td>
<td>8</td>
<td>285</td>
<td>GAG (E) → AAG (K)</td>
<td>missense</td>
<td>CCC(P)</td>
</tr>
<tr>
<td>HO-1-u-1</td>
<td>9</td>
<td>292-293</td>
<td>T insertion (frameshift)</td>
<td>1bp insertion</td>
<td>CGC(R)</td>
</tr>
<tr>
<td>HO-1-N-1</td>
<td>7</td>
<td>248</td>
<td>CGG (R) → CAG (Q)</td>
<td>missense</td>
<td>CGC(R)</td>
</tr>
<tr>
<td>TOS-1TY</td>
<td>8</td>
<td>280</td>
<td>rearrangement</td>
<td>rearrangement</td>
<td>CGC(R)</td>
</tr>
<tr>
<td>TOS-2FY</td>
<td>4</td>
<td>113</td>
<td>TTC (F) deletion (in-frame truncation)</td>
<td>3bp deletion</td>
<td>CCC(P)</td>
</tr>
<tr>
<td>TOS-3KS</td>
<td>7</td>
<td>248</td>
<td>CGG (R) → CAG (Q)</td>
<td>missense</td>
<td>CGC(R)</td>
</tr>
<tr>
<td>TOS-4CO</td>
<td>4</td>
<td>94</td>
<td>TCA (S) → TGA (OPA:STOP)</td>
<td>nonsense</td>
<td>CGC(R)</td>
</tr>
<tr>
<td>TOS-5MI</td>
<td>4</td>
<td>105</td>
<td>G deletion (frameshift)</td>
<td>1bp deletion</td>
<td>CCC(P)</td>
</tr>
<tr>
<td>TOS-6AS</td>
<td>4-5</td>
<td>59-184</td>
<td>rearrangement</td>
<td>rearrangement</td>
<td>—</td>
</tr>
</tbody>
</table>

Codon 248 mutations in the DNA binding domain were identified in 4 out of 15 cell lines. The genotype of the arginine code (72R) was detected in eight cell lines, and the genotype of the proline code (72P) was detected in six. The mutations of HSC-3, HSC-4, Ca9-22 and HOC313 were confirmed in a previous report. 25.
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was shifted. In SCC25, the serine code of codon 106 turned into an isoleucine code, and there after arginins was frame-shifted by the deletion of two bases of codon 209. In the SAS, the glutaminic acid code of codon 336 was replaced by Amber (Stop codon). The mutations of HSC-3, HSC-4, Ca9-22 and HOC313 were confirmed in a previous report25.

(2) SNP on codon 72

Single nucleotide polymorphism (SNP) has been previously reported26 on codon 72 of TP53 gene which codes either an arginine or proline. Eight cell lines showed the genotype which coded the arginine (72R) and six cell lines showed the genotype of proline (72P) (Table 2).

3. Expression level of p53 mRNA

The mRNA expression levels of the cell lines were determined by quantitative real-time PCR with cDNA. A standard curve was drawn by using p53 plasmid DNA as a standard, and the amount of p53 expression in each cell was measured. The value of the relative expression was calculated by dividing by the internal standard of GAPDH gene expression for comparison with a normal fibroblast cell line or with each other. In the novel cell lines, TOS-5MI showed at a low level, and it was high in TOS-2FY and TOS-1TY (Fig. 2).

4. Expression level of p53 protein

Next, the quantity of expressed p53 protein was measured using an ELISA in all of the cell lines (except TOS-3KS). TOS-1TY, TOS-2FY, HSC-4, Ca9-22, HOC313 and HO-1-N-1 showed high levels and SAS showed an intermediate level in the quantity of p53 (Fig. 3). These seven cell lines which showed p53 protein expression were classified as the p53(+) group, and the others which showed no expression were classified as the p53(−) group.

5. Cell invasion activity

The invasion activity of each cell line was evaluated with the basement membrane model in vitro. HSC-3 was measured as a positive control sample because it was confirmed that it caused lung metastasis in a mouse model in vivo35. TOS-2FY, TOS-5MI, HSC-4, SAS, SCC25, Ca9-22, HOC313, HO-1-u-1, HO-1-N-1, TOs-4CO and TOS-6AS each showed at nearly the same level as HSC-3. The levels of KB and TOS-1TY showed significantly lower than that of HSC-3 (Fig. 4-a). When the cell invasion activity was compared between the cell lines with and without the mutated p53 protein, the p53(+) group showed slightly higher levels than did the p53(−) group. Within the p53(+) group with the 72SNP, the cell invasion activity in the 72R type was higher than that in 72P type (Fig. 4-b).

6. The anti-cancer drug susceptibility

The anti-cancer drug susceptibility of each cell line was determined and compared with the KB cell line, which has previously been used to determine the efficacy of multiple anti-cancer drugs. Here, we examined...
the susceptibility against four kinds of anti-cancer drugs with different mechanisms of action, and which are used frequently. HSC-4, Ca9-22 and HO-1-u-1 showed a relatively high IC50 in DOX. Ca9-22, HOC313, HO-1-N-1 and HO-1-u-1 showed a relatively high IC50 of CDDP, while TOS-2FY, TOS-5MI and HO-1-u-1 showed a high IC50 of PEP. A high IC50 of VCR was shown in TOS-2FY and TOS-5MI (Fig. 5a-d-1).

The p53 group showed a slightly lower sensitivity to CDDP, but high sensitivity to DOX, VCR and PEP. Within the p53 group, the sensitivity of 72R type was apparently higher than that of the 72P, and a significant difference was recognized with type DOX and PEP.

7. Examination with a Saos-2 cell with recombinant introduced mutated TP53

To examine the effect of the mutated TP53 in cells with a matched background, mutated TP53 of R248Q and R273H was introduced independently into TP53 null Saos-2 cells, which were designated as Saos 248Q and Saos 273H cells, respectively. The expression of the introduced mutated TP53 was confirmed in both the Saos 248Q and Saos 273H cells (Fig. 6a-1,2). Saos neo introduced with the vector only, and in Saos273H, showed that the cell invasion activity was low compared with that of HSC-3, but that of Saos 248Q was higher than that of Saos neo (Fig. 6). Although IC50 of the anti-cancer drugs differed according to the drug, Saos273H showed a resistant tendency in CDDP, but both transformants were obviously susceptible to DOX, PEP, and VCR compared to Saos neo.

Discussion

In this report, we examined the relationship between variation in the TP53 mutation and the characteristics of OSCC by using cultured cells. While we recognized that the in vitro culture may not retain all the phenotypes seen in vivo, it is still of value to culture tumor cells in order to examine and better understand the functional characteristics of living cells, as is demonstrated by the many cell biological discoveries that were potentiated by the use of cultured cells in cancer research. In the present study, we sought to establish OSCC cell lines from cells derived from OSCC cases from our and other facilities, and we successfully identified the TP53 gene mutation in all the strains (except KB) established as cell lines. This suggests the possibility that the cancer cells with TP53 mutations were selected for during the establishment. The TP53 gene is thought to be an important suicide gene at a DNA injury site which eliminates the cell by inducing apoptosis. Therefore, the p53 protein which bears this function is not usually detected in normal unstressed cells. However, when the TP53 gene mutates, the mutated p53 protein is excessively produced or accumulated, thereby compromising apoptosis and leading to abnormal or malignant cell growth. In our hands, however, not all OSCC cells showed overexpression, with about half of them lacking this expression (Fig. 3).
(a-1) A staining image of each cell line which passed a membrane with extracellular matrix and observed to confirm the evaluation in Fig. 4 (a-2).

(a-2) The eluted strains was measured at OD 560. The value was compared with HSC-3 and KB and TOS-1TY were judged to be significantly low.

(b) A comparison in the presence of the mutated p53 protein expression and codon 72 SNP. The p53(+) group tended to show a slightly high level. The 72R type showed a higher tendency when compared in 72 SNP among the p53(+) group.
In any case, our research should be useful for determining the relationship between malignancy, drug sensitivity, and the presence of or deficiency in the p53 mutant.

Codons 175, 245, 248, 273, and 282 have been listed as hot spots for TP53 gene mutation. A codon 248 mutation was found in 4 of 15 cell lines in the present study. Since the area containing this hot spot is considered part of the core domain (DNA binding domain) and is thought to be an important region for transcription factor functions, the hot-spot mutants of the p53 protein are likely to at least contribute to abnormal transcriptional activity of the target genes as well as to cell malignancy. Although the level of mRNA expression according to real-time PCR and the amount of the p53 protein according to an ELISA was consistent, this did not correlate with the observation for KB, TOS-1TY, or SCC25 (Figs. 2 and 3). There was excessive protein in TOS-1TY, even though the level of mRNA expression was not excessive. In cancer cells, the pathology seems to be related to accumulation because the resolution was not excessive. In cancer cells, the pathology seems to be related to accumulation because the resolution was not excessive. In cancer cells, the pathology seems to be related to accumulation because the resolution was not excessive. In cancer cells, the pathology seems to be related to accumulation because the resolution was not excessive. In cancer cells, the pathology seems to be related to accumulation because the resolution was not excessive.

Regarding anti-cancer drug sensitivity, some studies have reported that the mutated p53 (+) showed low sensitivity to most drugs, although it showed a similar or higher sensitivity to drugs that work in microtubule polymerization obstruction, such as VCR and Taxol. However, these findings may vary by cell type, mutation variety, and the study design, and much more data must be collected to better understand these phenomena.

In the TP53 gene, SNP with an amino acid substitu-
tion at codon 72 is known. It has also been reported that an arginine or proline on codon 72 will influence both of the wild type p53 and the mutated p53. The wild type p53 localizes to the mitochondria and increases the anti-cancer drug sensitivity by inducing apoptosis by discharging cytochrome c from the mitochondria to the cytoplasm, and this localization has been observed strongly for the 72R type of p53. While there are various reports suggesting a relationship between the malignant phenotype and the influence of codon 72 SNP, there is no consensus in the literature. Thus, we examined the susceptibility of OSCC lines to four common anti-cancer drugs, each of which has a different mechanism of action, and found that the 72R type mutated p53 protein was more sensitive to each drug than was the 72P type, with significant differences recognized in DOX and PEP (Fig. 5). However, Sullivan et al. showed that the wild type p53 of the 72R variety was more sensitive to cisplatin than was the 72P variety in a head and neck SCC cell line, and that the mutated p53 showed the inverse of this sensitivity pattern. This phenomenon was thought to indicate that the mutated p53 of the 72R type was bound to the p53 family, p73, and inhibits the induction of apoptosis, but it was not determined in other drugs than cisplatin. Thus, sensitivity varies not only with the presence of the p53 mutation, but also with the drug type and the variety of the individual mutation. While the difference was not significant, the 72R variety showed a higher tendency towards cell invasion activity. Further studies should investigate the influence on cancer control mechanisms of the cell invasion activity as a function of the variety (72P/R) and the TP53 mutation type.

In our study of these OSCC cell lines, the hottest mutation of TP53 was on codon 248, which is also known to show the highest frequency in all cancers in the database and the next-most frequent mutation was on codon 273. The pathogenicity of these mutations with a high frequency is considered to be especially high. We introduced these two TP53 mutations into the p53 null Saos-2 cell line independently to clarify the characteristics of the mutations using a fixed cell background. The sensitivity to anti-cancer drugs of each mutation was different as predicted, depending on both the TP53 mutation type and drug type. The sensitivity of both transformants increased a little in DOX and PEP, and Saos273H showed a resistance tendency opposite to that of CDDP. The sensitivity of the VCR for both transformant types was clearly high. However, the phenotype and the sensitivity probably differ as a function of the cell type; Saos-2 is an osteogenic sarcoma and it should be tested on the OSCC cell lines in the same manner as we tested.

In terms of cell invasion activity, the Saos 248Q line showed a permeation tendency when compared with the control Saos neo, and this was confirmed in OSCC with codon 248 mutated cell lines (HSC-4, Ca9-22, HO-1-N-1), and the host of TOS-3KS was also diagnosed as N1. Therefore, it was thought that the 248Q mutation of p53 contributed to cell malignancy, although the detailed effects of other mutations must also be considered. While it may one day be possible to predict the characteristics of OSCC, such as its invasion activity, metastasis, and sensitivity to drugs, based on an analy-
sis of the TP53 mutation and SNP on codon 72, further studies that account for a variety of variables will be necessary to identify all the relevant factors.

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