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ORIGINAL ARTICLE

Differential expression of p53, p63 and p73 protein and mRNA for DMBA-induced hamster buccal-pouch squamous-cell carcinomas

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Summary

Abnormalities in the *p53* gene are regarded as the most consistent of the genetic abnormalities associated with oral squamous-cell carcinoma. Two related members of the *p53* gene family, *p73* and *p63*, have shown remarkable structural similarity to *p53*, suggesting possible functional and biological interactions. The purpose of this study was to investigate the differential expression of *p73*, *p63* and *p53* genes for DMBA-induced hamster buccal-pouch squamous-cell carcinoma. Immunohistochemical analysis for protein expression and reverse transcriptase-polymerase chain reaction (RT-PCR) for mRNA expression were performed for 40 samples of hamster buccal pouches, the total being separated into one experimental group (15-week DMBA-treated; 20 animals) and two control groups (untreated and mineral oil-treated; 10 animals each). Using immunohistochemical techniques, nuclear staining of p53 and p73 proteins was detected in a subset of hamster buccal-pouch tissue specimens treated with DMBA for a period of 15 weeks, whereas p63 proteins were noted for all of the 20 hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks as well as for all of the untreated and mineral oil-treated hamster buccal-pouch tissue specimens. Differential expression of p63, p73 and p53 protein for the experimental group was as follows: p63+/p73+/p53+ ($n = 14$; 70%); p63+/p73+/p53– ($n = 2$; 10%); p63+/p73–/p53– ($n = 4$; 20%) and p63+/p73–/p53– (untreated [$n = 10$] and mineral oil-treated mucosa [$n = 10$]; 100% each). Upon RT-PCR, ΔNp63mRNA was detected within all of the 20 hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks, whereas expression of TAp63 was not detected. Furthermore, p73 mRNA was identified for 16 of the hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks, whereas p53 mRNA was noted for 14 15-week DMBA-treated pouches. The proportional (percentage) expression of ΔNp63, p73 and p53 mRNA for the hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks was noted to be consistent with the findings using immunohistochemical techniques. A significant correlation between p53, p63 and p73 expression (protein and mRNA) was demonstrated for the hamster buccal-pouch carcinoma samples. Our results indicate that both p73

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and p63 may be involved in the development of chemically induced hamster buccal-pouch carcinomas, perhaps in concert with p53.

Keywords
DMBA-carcinogenesis, hamster, p53, p63, p73

About two decades subsequent to the discovery of the *p53* tumour-suppressor gene, two related genes (*p73* and *p63*) have been cloned giving rise to the notion of a p53 family of genes (Kaghad *et al*. 1997; Osada *et al*. 1998; Trink *et al*. 1998; Yang *et al*. 1998; Kaelin 1999). Due to the significant structural similarity of these two genes with *p53*, it would seem not unreasonable to expect that their function would be similar to *p53* in terms of tumour suppression, induction of apoptosis and/or cell-cycle control, although it has been revealed that the relationship between this family of genes is much more complex than may have been first thought. Structurally, p53 features a single promoter with three conserved domains, namely, the transactivation (TA) domain, the DNA-binding domain and the oligomerization domain. By contrast, p63 and p73 each feature two promoters, resulting in two different types of protein products: those containing the TA domain (TAp63 and TAp73) and those lacking the TA domain (ΔNp63 and ΔNp73) (Trink *et al*. 1998; Yang *et al*. 2000). Furthermore, both *p63* and *p73* genes undergo alternative splicing at the COOH terminus, giving rise to three isotypes (α, β and γ) (Kaghad *et al*. 1997; Yang *et al*. 1998; Yamaguchi *et al*. 2000). These various isotypes have previously been reported to possess either similar or opposite functions to those of p53-related transcription factors, depending upon which particular isotypes are expressed (Jost *et al*. 1997). In general, the TAp63 (TAp73) isotypes might behave like p53 because they, reportedly, transactivate various p53 downstream targets, induce apoptosis and mediate cell-cycle control. The ΔNp63 (ΔNp73) isotypes, however, have been shown to display opposing functions to the TAp63 (TAp73) isotypes, including acting as oncoproteins (Hibi *et al*. 2000; Ratovitski *et al*. 2001; Patturajan *et al*. 2002; Stiewe *et al*. 2002; Zaika *et al*. 2002).

The hamster buccal-pouch mucosa constitutes one of the most widely accepted experimental models for oral carcinogenesis investigation (Gimenez-Conti & Slaga 1993). Despite anatomical and histological variations between hamster-pouch mucosa and human buccal tissue, experimental carcinogenesis protocols for the former are able to be devised so as to induce premalignant changes and carcinomas there that resemble those that take place during analogous development in human oral mucosa (Morris 1961).

As discussed above, both p73 and p63 share remarkable sequence homology with p53, indicating possible functional and biological interactions, although the differential expression of p73, p63 and p53 for DMBA-induced hamster buccal-pouch squamous-cell carcinomas does not yet appear to be completely understood. Therefore, the aim of this study was to investigate the expression of p73, p63 and p53 protein and mRNA for DMBA-induced hamster buccal mucosa squamous-cell carcinomas.

Materials and methods

Animals

Outbred, young (6-week-old), male, Syrian golden hamsters (*Mesocricatus auratus*; 40 animals) were purchased from the National Science Council Animal Breeding Center, Taipei, ROC, weighing approximately 100 g each at the commencement of the experiment. These animals were randomly divided into one experimental group (20 animals) and two control groups (10 animals per group). The animals were housed under constant conditions (22 °C and a 12-h light/dark cycle) and supplied with tap water and standard Purina laboratory chow *ad libitum*. Appropriate animal care and an approved experimental protocol ensured humane treatment, and all procedures were conducted in accordance with the guidelines promulgated by the NIH Guide for the Care and Use of Animals. After allowing the animals for 1 week of acclimatization to their new surroundings, both pouches from all of the animals from the experimental groups were painted with a 0.5% DMBA solution at 9 a.m. on Monday, Wednesday and Friday of each week, using a no. 4 sable hair brush. Bilateral pouches from each animal from the mineral-oil group were similarly treated with mineral oil. Approximately, 0.2 ml of the appropriate solution was applied topically to the medial walls of both pouches at each painting. The untreated group of 10 animals remained untreated throughout the experiment.

At the end of 15 weeks (3 days subsequent to the last treatment), in order to avoid the influence of diurnal variation, all of the participating animals were simultaneously and humanely sacrificed at 9 a.m., by the administration of a lethal dose of diethyl ether (Lin & Chen 1997). The animals'

Table 1 Oligonucleotide primers used to amplify p53, ΔNp63, TAp63, p73 and β-actin cDNAs

Oligonucleotide primers	Sequences	Polymerase chain reaction products
p73 sense	5′-CTCCCCGCTCTTGAAGAAAC-3′	180 bp
p73 antisense	5′-GTTGAAGTCCCTCCCGAGC-3′	
ΔNp63 sense	5′-CAGACTGAATTTAGTGAG-3′	400 bp
ΔNp63 antisense	5′-AGCTGATGGTTGGGGCAC-3′	
TAp63 sense	5′-ATTCCCAGAGCACACAG-3′	600 bp
TAp63 antisense	5′-AGCTCATGGTTGGGGCAC-3′	
p53 sense	5′-CTGAGGTTGGCTCTGACTGTACCACCATCC-3′	370 bp
p53 antisense	5′-CTCATTCAGCTCTCGGAACATCTCGAAGCG-3′	
β-actin sense	5′-AACCGCGAGAAGATGACCCAGATCATGTTT-3′	350 bp
β-actin antisense	5′-AGCAGCCGTGGCCATCTCTTGCTCGAAGTC-3′	

pouches were exposed by dissection and examined grossly. Both pouches were then excised. A portion of the pouch tissue was immediately frozen in liquid nitrogen for subsequent RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR) reaction investigation, whilst another portion was fixed in 10% neutral-buffered formalin solution for about 24 h, dehydrated in a series of ascending-concentration alcohol solutions, cleared in xylene and embedded in paraffin for immunohistochemical studies.

Immunohistochemistry

Following tissue sectioning, staining was performed using a standard avidin-biotin peroxidase complex (ABC) method (Hsu *et al*. 1981). The primary antibodies used were: a polyclonal antibody raised against p73 (catalogue number sc-7957, 1 : 100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), a monoclonal antibody for p63 (clone 4A4, 1 : 100 dilution; Santa Cruz Biotechnology) and a monoclonal antibody for p53 (DO-7, 1 : 100 dilution; Novocastra, Newcastle, UK). Rabbit polyclonal antibodies to p73 were raised against a recombinant protein corresponding to amino acids 1–80, mapping at the amino terminus of human-origin p73. The p63 antibody was raised against amino acids 1–205 mapping at the amino terminus of ΔNp63. According to the manufacturer's specifications, these antibodies specific to p73 and p63 react broadly with all known p73 and p63 variants of human, rat and mouse origin, respectively, as determined by Western blotting and immunohistochemistry (including paraffin-embedded sections) techniques (Santa Cruz Biotechnology). The specificity of the anti-p63 antibody has been previously demonstrated by a variety of immunoblotting experiments, as well as by analogous immunohistochemical-staining studies of mouse tissues from which the *p63* gene had been deleted (Yang *et al*. 1998, 1999). The p53 DO-7 antibodies detected both wild-type and mutant forms of p53 (Vojtesek *et al*. 1992).

Tissue sections were mounted on gelatin-chrome alum-coated slides. Subsequent to deparaffinization in xylene (twice) and rehydration with a descending-concentration ethanol series (absolute, 95%, 70% and 30% ethanol, and then water), tissue sections were microwave treated three times (5 min each) in citrate buffer (10 mM; pH = 6.0) in order to retrieve antigenicity. The tissue sections were then treated in H_2O_2-methanol (0.3%) and normal goat serum (10%; Dako; Santa Barbara, CA, USA). All sections were subsequently incubated with the primary antibodies (p73 and p63), at room temperature for 60 min each, whilst for the antibody for p53, the exposure was at 4 °C overnight. These sections were then incubated for a further 30 min at room temperature in the presence of biotinylated goat anti-rabbit immunoglobulin G (IgG) for p73, and biotinylated goat anti-mouse IgG for p63 and p53 (both 1 : 100; Vector, Burlingame, CA, USA) and then for a final 30 min with ABC (Dako). The peroxidase-binding sites were visualized as brown reaction products of the benzidine reaction. The sections were subsequently counterstained with haematoxylin. Positive and negative controls were used for each experiment. As p73, p63 and p53 are nuclear proteins, only nuclear positivity was assessed. Immunohistochemical staining was classified as negative if staining was apparent for l0% of the cells or less, or positive where more than 10% of cells present were positively stained.

Reverse transcription-polymerase chain reaction

Total RNA was extracted by homogenizing the pouch tissue specimens in guanidium isothiocyanate followed by ultracentrifugation in caesium chloride, as described previously (Chomczynski &

Sacchi 1987). The RNA concentration was determined by way of the sample's optical density at a wavelength of 260 nm (by using an OD260 unit equivalent to 40 μg/ml of RNA).

Isolated total RNA (1 μg) was reverse-transcribed to cDNA in a reaction mixture (with a final volume of 20 μl) containing $MgCl_2$ (4 μl; 5 mM), ×10 reverse transcription buffer [2 μl; 10 mM Tris–HCl (pH = 9.0), 50 mM KCl, 0.1% Triton® X-100], deoxyribonucleoside triphosphate (dNTP) mixture (2 μl; 1 mM each), recombinant Rnasin® ribnonuclease inhibitor (0.5 μl; 1μ/μl), avian myeloblastosis virus (AMV) reverse transcriptase (15 units; High Conc.; 15μ/μg) and oligo(dT)15 primer (0.5 μg; Promega, catalogue number A3500, WI, USA). The reaction mixture was incubated for 15 min at 42 °C. The AMV reverse transcriptase was inactivated by heating for 5 min at 99 °C and then incubating at 0–5 °C for a further 5 min.

All oligonucleotide primers were purchased from Genset corp. (La Jolla, CA, USA). The primer pairs were chosen from the published cDNA sequences of p53 (Chen *et al.* 2003a), p63 (TA and ΔN isoforms; Glickman *et al.* 2001), p73 (Cai *et al.* 2000) and β-actin (Chen *et al.* 2003a). Oligonucleotide primers used for PCR reactions are summarized in Table 1. The 20-μl first-strand cDNA synthesis reaction product obtained from the reverse transcriptase reaction was diluted to 100 μl with nuclease-free water. The PCR amplification reaction mixture (with a final volume of 100 μl) contained diluted, first-strand cDNA reaction product (20 μl; <10 ng/μl), cDNA reaction dNTPs (2 μl; 200 μM each), $MgCl_2$ (4 μl; 2 mM), ×10 reverse transcription buffer (8 μl; 10 mM Tris–HCl, pH = 9.0, 50 mM KCl, 0.1% Triton® X-100), upstream primer (50 pmol), downstream primer (50 pmol) and Taq DNA polymerase (2.5 units; Promega, catalogue number M7660).

The PCR steps were carried out on a DNA thermal cycler (TaKaRa MP, Tokyo, Japan). Thermocycling conditions included denaturing at 94 °C for 1 min (one cycle), then denaturing at 94 °C (1 min), annealing at 55 °C (1 min) for p73, 52 °C (1 min) for both ΔNp63 and TAp63, 55 °C (1 min) for p53 or at 60 °C (1 min) for β-actin, and extending at 72 °C (1 min) for 30 cycles and a final extension at 72 °C for 7 min. The β-actin primers were utilized as positive controls. Negative controls, i.e. those conducted in the absence of RNA and reverse transcriptase, were also performed. Amplification products were analysed by electrophoresis in a 2% agarose gel along with the relevant DNA molecular weight marker (Boehringer, Mannheim, Germany) and stained with ethidium bromide. The PCR products were visualized as bands with a UV transilluminator. Photographs were taken with a Polaroid DS-300 camera. The PCR products were then sequenced to confirm their identities using a T7 Sequenase version 2.0 kit (Amersham International, Little Chalfont, UK).

Results

Gross observation and histopathology

Gross and histopathological changes amongst the 15-week DMBA-treated pouches were similar to those described in our previous study (Chen *et al.* 2002a). Squamous-cell carcinomas with a 100% tumour incidence were apparent for all of the 15-week DMBA-treated pouches. The mineral oil-treated and untreated pouches revealed no obvious changes associated with such treatment.

Immunohistochemical staining

Using immunohistochemical techniques, nuclear staining of p53 and p73 proteins (Figure 1a,b) was detected for a subset of hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks, whereas p63 proteins (Figure 1c) were noted for all of the 20 hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks as well as for all of the untreated and mineral oil-treated hamster buccal-pouch tissue specimens (Figure 1d). Differential expression of p63, p73 and p53 protein for the experimental groups was as follows: p63+/p73+/p53+ ($n = 14$; 70%); p63+/p73+/p53– ($n = 2$; 10%); p63+/p73–/p53– ($n = 4$; 20%) and p63+/p73–/p53– (untreated and mineral oil-treated mucosa; 10 animals, 100% for each).

Nuclear staining of p63 was noted in the basal layers of the untreated and mineral oil-treated hamster buccal-pouch mucosa (Figure 1d), whilst, by contrast, p73 and p53 expression was not noted in the untreated and mineral oil-treated pouch mucosa. For the carcinoma samples, both p73 and p63 immunoreactivity was chiefly observed for the less-differentiated cells located at the periphery of carcinomatous clusters (Figure 1b,c). For p53 staining, positive labelling was demonstrated for some cells in the upper layers of the tumour islands (Figure 1a). In addition, a significant correlation was demonstrated between p53, p63 and p73 immunoexpression in the 15-week DMBA-treated hamster buccal-pouch carcinomas.

Reverse transcription-polymerase chain reaction

Upon RT-PCR, ΔNp63mRNA was detectable as a band corresponding to a 400-bp PCR product for all of the 20 hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks, whereas expression of TAp63 was not detected (Figure 2). p73 mRNA was identified as a band corresponding to a 180-bp PCR product and was observed for 16 of the hamster buccal-pouch tissue specimens treated with DMBA

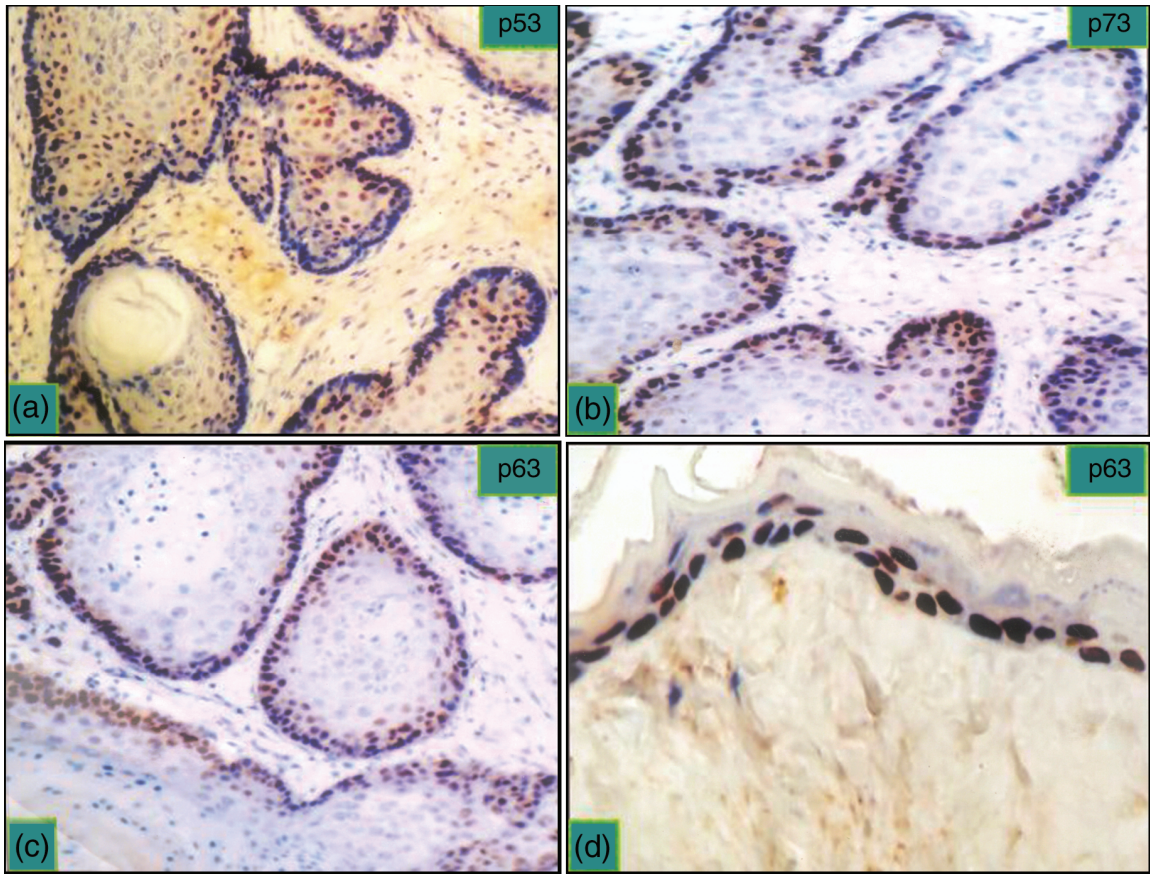

Figure 1 Representative section of a buccal carcinoma specimen revealing p53 (a), p73 (b) and p63 (c) immunoreactivity, chiefly observed for the less-differentiated cells located at the periphery of tumour islands. Note that positive p53 staining was also demonstrated for some cells in the upper layers of the tumour islands (a). Nuclear staining of p63 was noted in the basal layers of the untreated and mineral oil-treated hamster buccal-pouch mucosa tissue (d) (ABC stain, ×100 magnification).

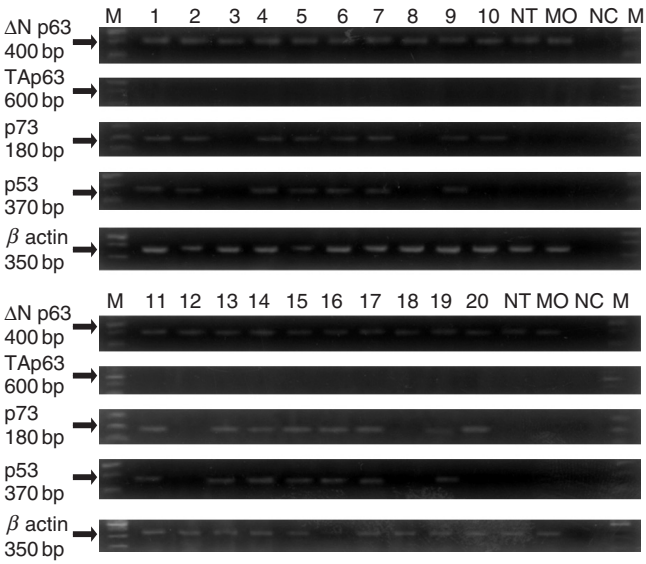

Figure 2 Expression of p63 (ΔN and TA isotypes), p73 and p53 mRNA in hamster buccal-pouch carcinomas using reverse transcription-polymerase chain reaction (RT-PCR). A band of a 400-bp PCR product corresponding to ΔNp63mRNA is observed for all the hamster buccal-pouch tissue specimens treated with DMBA over a period of 15 weeks (lanes 1–20), whereas no specimens of buccal-pouch carcinoma reveal a 600-bp PCR product corresponding to TAp63mRNA (lanes 1–20). A band of a 180-bp PCR product corresponding to p73mRNA may be observed for 16 specimens (lanes 1,2, 4–7, 9–11, 13–17, 19 and 20), whereas a band of a 370-bp PCR product corresponding to p53mRNA may be observed for 14 specimens (lanes 1, 2, 4–7, 9, 11, 13–17 and 19). A band of PCR product (400-bp) corresponding to ΔNp63mRNA is observed for all the untreated (lane NT) and mineral oil-treated (lane MO) specimens. No bands for p63 (ΔN and TA isotypes), p73 and p53 mRNA are noted for untreated (lane NT), mineral oil-treated (lane MO) and the negative control (lane NC) samples. All samples (lanes 1–20, NT, MO) apart from the negative-control sample (lane NC) reveal bands of β-actin (350-bp). Lane M is the DNA molecular weight marker.

for 15 weeks (Figure 2). p53 mRNA was found as a band corresponding to a 370-bp PCR product and was noted for 14 15-week DMBA-treated pouches (Figure 2) The proportional (percentage) expression of ΔNp63 (20/20, 100%), p73 (16/20, 80%) and p53 (14/20, 70%) mRNA for the hamster buccal-pouch tissue specimens treated with DMBA for 15 weeks was noted to be consistent with the findings using immunohistochemistry. In addition, there was a significant association detected between p53, p63 and p73 mRNA expression. The ΔNp63mRNA was detectable for all of the untreated and mineral oil-treated hamster buccal-pouch tissue specimens, whereas expression of TAp63 was not detected (Figure 2). On the other hand, no p73 and p53 mRNA presence was noted for the tissue deriving from the untreated animals, the mineral oil-treated tissues or the negative-control samples (Figure 2). All samples, apart from the negative-control samples, revealed bands of β-actin (350 bp; Figure 2). On direct sequencing, the 180-bp and 370-bp bands were confirmed to be parts of the *p73* and *p53* genes, respectively; also, 400-bp band was part of the *ΔNp63* gene. No mutations were found for those primers to be selected for this study.

Discussion

Although the expression of p73, p63 and p53 in hamster buccal-pouch carcinomas has been investigated previously by our group (Chen *et al*. 2002b, 2003a, 2003b) as also other workers (Chang *et al*. 1996; Gimenez-Conti *et al*. 1996), it would appear, to the best of our knowledge, that the differential expression for all of the p53 homologues, namely, p73, p63 and p53 for chemically induced experimental oral carcinomas has not been previously reported. In this study, the differential expression of p73, p63 and p53 protein and mRNA was characterized for a subset of DMBA-induced squamous-cell carcinomas arising from hamster buccal-pouch buccal mucosa, with further elucidation of the expression for all of the p53 homologues in an experimental oral carcinoma model. Furthermore, in the present study, a significant correlation was demonstrated for the p53, p63 and p73 protein and mRNA for DMBA-induced hamster buccal-pouch carcinomas, indicating that p73 and p63 may participate in oral experimental carcinomas, in concert with p53.

As mentioned earlier, the *p63* gene can be expressed into at least six protein isotypes, which are divided into two groups, those containing the TA domain (TA isotypes) and those that do not (ΔN isotypes) (Little & Jochemsen 2002). These various p63 isotypes have been reported to possess either similar or opposite functions to those of p53-related transcription factors, giving rise to the possibility that p63 could act either as a p53-like tumour-suppressor gene, or as a dominant oncogene, depending upon which particular isotypes are expressed (Jost *et al*. 1997). Immunohistochemistry using the 4A4 antibody, however, was not able to absolutely confirm which isotypes (TA or ΔN) were implicated in experimental oral carcinogenesis. The presence of TAp63mRNA within skeletal muscle tissue, in the absence of staining with the 4A4 antibody, has been previously reported (Di Como *et al*. 2002), indicating that this antibody may not necessarily identify all isotypes of p63 using immunohistochemical techniques. In order to elucidate which isotypes of p63 were expressed in the hamster buccal-pouch mucosa, RT-PCR was performed using isotype-specific primers. The ΔNp63mRNA was easily detectable within all carcinomatous pouch tissue specimens as well as the untreated and mineral oil-treated pouch mucosa tissue, whereas expression of TAp63 was not able to be detected in any of these tissue specimens. No variation of ΔNp63mRNA at the expression level was recognized between normal and carcinomatous pouch tissue. On the basis of this finding, we were able to conclude that ΔNp63 is the major isotype of p63 in the hamster buccal-pouch mucosa. The results of the current study, using a RT-PCR assay, confirm and expand our earlier immunochemical results using the 4A4 antibody (Chen *et al*. 2003b), indicating that the p63 proteins recognized by the 4A4 antibody are actually the ΔN isotype of p63.

Immunohistochemical detection of p73 protein in the early stages of DMBA-induced hamster buccal-pouch squamous-cell carcinogenesis has been reported in our previous study (Chen *et al*. 2002b). In the current study, we further demonstrated that both the p73 protein and the associated mRNA have been observed in DMBA-induced hamster buccal-pouch carcinomas. In addition, ΔNp73 has been implicated with possible oncogenic potential in a recent *in vitro* human tumour study (Ishimoto *et al*. 2002) and has been overexpressed in many human tumour tissue specimens, but not so for corresponding normal tissue (Zaika *et al*. 2002). Thus, further study is clearly warranted in order to determine which isotypes of p73 (TA or ΔN) contribute to the underlying mechanism of experimental oral carcinogenesis.

To the best of our knowledge, despite an extensive search of the available literature, it would appear that mutation of the *p73* and *p63* genes has rarely been detected in human cancers (Han *et al*. 1999; Jost *et al*. 1997 Hagiwara *et al*. 1999). Judging from these reported results, it remains possible that wild-type p73 and p63, but not the corresponding mutant forms, may have been expressed in the cancerous pouch keratinocytes detected in the present study, alluding to the possibility that both p73 and p63 may play an oncogenic role in experimental oral carcinogenesis through the expression of wild-type forms of these proteins rather than as tumour suppressors via the mutant forms. Identifying the specific mechanism

reflected in the correlation between p73, p63 and p53 protein and mRNA expression for pouch cancerous keratinocytes would appear critical if we are to fully elucidate the role of p73 and p63 in experimental oral oncogenesis. This is possibly due to the disruption of normal p53 function, resulting in a compensatory upregulation of p73 and p63 expression, such that either the production of mutant p53 or a reduction in p21WAF/CIP1 may also trigger an increase in the expression of p73 and p63 (Jost *et al*. 1997).

In this study, p73 and p63 protein and the corresponding mRNA were expressed simultaneously and were positively correlated with each other, suggesting a synergistic effect with respect to tumour development in the chemically induced experimental carcinoma model. Our data is also consistent with previous observations for human oral squamous-cell carcinomas (Faridoni-Laurens *et al*. 2001; Choi *et al*. 2002; Weber *et al*. 2002; Chen *et al*. 2003c). Noteworthy also is our observation that expression of either p73 and p63 (protein and mRNA) (p63+/p73+/p53–; $n = 2$) or p63 (protein and mRNA) only (p63+/p73–/p53–; $n = 4$) has been previously demonstrated for six p53-negative lesions, indicating that independent or complementary biological functions for these three genes may, despite possible modest contribution, also exist for chemically induced hamster buccal-pouch carcinogenesis.

Our immunohistochemical analysis study indicates that p73 and p63 proteins are found in less-differentiated cells at the periphery of carcinomatous tissues. These observations suggest a relationship between p73 and p63 (protein and mRNA) and the differentiation of hamster buccal-pouch mucosa. These findings appear to be consistent with the results of similar investigations pertaining to human oral squamous-cell carcinomas (Faridoni-Laurens *et al*. 2001; Choi *et al*. 2002; Chen *et al*. 2003c).

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