Changes in the expression of gap junction proteins (connexins) in hamster tongue epithelium during wound healing and carcinogenesis

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Introduction

Gap junctions are specialized cell–cell junctions that form intercellular channels and mediate the direct transfer of low molecular weight (<1000 kDa) metabolites and ions, including second messengers such as cyclic adenosine monophosphate (cAMP*), inositol trisphosphate and Ca2+, between adjacent cells in contact. Gap junctional intercellular communication (GJIC) is considered to play important roles in control of cell growth, differentiation, maintenance of homeostasis and morphogenesis. Gap junctions are composed of connexins (Cx), which are coded for by a multigene family consisting of at least 12 members in rodents (1–3).

Keratinocytes in the squamous epithelium are connected by gap junctions. Gap junctions in keratinocytes were detected electron microscopically (4–6) and functionally (7,8). We have previously demonstrated that Cx26 and Cx43 are expressed in keratinocytes in mouse epidermis, but that in most cases localization of the two kinds of Cx is different, i.e. Cx26 is expressed in keratinocytes in the granular layer and in the upper part of the squamous layer, whereas Cx43 is localized in keratinocytes in the basal layer and in the lower part of the squamous layer, suggesting that the modulation of Cx expression from Cx43 to Cx26 is closely related to terminal differentiation of keratinocytes (9).

Modulation of gap junctions has been studied fairly extensively in hepatocytes in vivo during controlled cell growth after 70% partial hepatectomy (10–15) and autonomous cell growth during carcinogenesis (3,15–22). GJIC and Cx expression have also been shown to be altered during wound healing of endothelial cells in vitro (23–25). However, only a limited amount of information exists on what changes in expression and localization of Cxs in keratinocytes in squamous epithelium occur during wound healing, in which migration, proliferation and differentiation of keratinocytes takes place in a coordinated manner, and carcinogenesis, during which tumor cells acquire autonomous growth, except for a few recent papers on rat epidermal wound healing (26) and mouse skin carcinogenesis (27,28).

In the present study, we performed immunofluorescence and in situ hybridization studies of hamster tongue epithelium to answer the following questions: (i) in normal squamous epithelium, is different localization of Cx26 and Cx43 proteins affected by a variety of factors, such as wound healing and carcinogenesis? (ii) what changes in the expression and localization of Cx mRNAs and proteins occur during wound healing?: (iii) what changes in the expression and localization of Cx mRNAs and proteins occur during oral epithelium carcinogenesis?

Materials and methods

Animals and treatments

Seven-week-old male Syrian hamsters were purchased from Charles River Japan Inc. (Atsugi, Japan). The tongue undersurface was excised under ether anesthesia and used as intact hamster tongues.

Wounding and preparation of wound tissues

Twenty-eight male Syrian hamsters, 7 weeks old, were used. Under anesthesia, standardized excisional wounds were made perpendicularly to the long axis of the tongue undersurface with scalpels. Each wound was 2–3 mm in length and about 0.5 mm in depth. Animals were killed under...
ether anesthesia and wounds were harvested at 6 h, 12 h, 18 h, 1 day, 3 days, 5 days and 7 days after wounding. Four animals were used for each time point.

**Tongue carcinogenesis**

We used the carcinogenesis protocol described by Fujita et al. (29) with modifications to induce tongue papillomas and carcinomas in male Syrian hamsters. Two hundred microliters of 0.1% dimethylbenz[a]anthracene (DMBA) in acetone were applied to the undersurface of tongues of 7-week-old male Syrian hamsters and the application was continued three times a week for up to 24 weeks. Each animal was examined for tumor appearance weekly.

Bromodeoxyuridine (BrdU; 100 mg/kg body weight; DAKO, Copenhagen, Denmark) in 1 ml of phosphate-buffered saline (PBS) was injected i.p. into all animals, including non-treated, wounded and carcinogen-treated hamsters at 1 h before being killed. At the time of killing under ether anesthesia, intact, wound, papilloma and carcinoma tissues were immediately frozen in Tissue Tek O.C.T. compound (Miles Inc., Elkhart, IN) for immunofluorescence study. Additional portions were quick-frozen in liquid nitrogen and stored at −70°C for RNA and protein extractions.

**Northern blot analysis**

Northern blot analysis for Cx26, Cx32 and Cx43 in hamster tongue epithelium and rat heart and liver was performed using digoxigenin (DIG)-labeled RNA probes prepared from rat Cx26 (30), Cx32 (31) and Cx43 (32) cDNAs as described by Kamibayashi et al. (9). Hybridization, washing and chemiluminescent detection were carried out following the DIG luminescent protocol (33).

**Western blot analysis**

Western blot analysis for Cx26 and Cx43 in hamster tongue epithelium and mouse heart and liver was performed using digoxigenin (DIG)-labeled RNA probes for Cx26 and Cx43. Alkali-treated tissue homogenates of hamster tongue epithelium, rat heart and liver. Total RNA from hamster tongue epithelium was examined for Cx43, Cx32 and Cx26 using specific DIG-labeled RNA probes. Lane 1, hamster tongue epithelium; lane 2, rat heart; lane 3, rat liver. Under stringent conditions, clear bands with the Cx43 probe (A) and the Cx26 probe (C) were detected in hamster tongue epithelium. No bands were observed in hamster tongue epithelium with the Cx32 probe (B). The lower halves of the figure show ethidium bromide staining of ribosomal RNAs before transfer to membranes.

**Immunofluorescence**

Double immunofluorescent staining for Cx26 and Cx43 was performed using a rabbit anti-rat Cx26 polyclonal antibody (34) and a mouse anti-Cx43 monoclonal antibody (Zymed Laboratories, Inc., San Francisco, CA) as described by Kamibayashi et al. (9).

**In situ hybridization**

Intact, wound, papilloma and carcinoma tissues were fixed in 4% paraformaldehyde in PBS for 8 h at 4°C. The samples were dehydrated in ethanol series, embedded in paraffin, and sectioned at 5 µm on silanized slides (DAKO). Subsequently, sections were deparaffinized in benzene, rehydrated, pretreated with 10 µg/ml proteinase K, and then with 0.1 M triethanolamine buffer (pH 8.0) containing 0.25% acetic anhydride. The sections were hybridized with DIG-labeled antisense RNA probes for Cx26, Cx32 or Cx43 for 16 h at 50°C. Stringent washes were performed for 30 min at 50°C with 50% formamide in 2 × SSC and the slides were treated with 20 µg/ml RNase A (Boehringer Mannheim) for 30 min at 37°C. The sections were incubated with an anti-DIG antibody coupled to alkaline phosphatase (1/1000 dilution; Becton Dickinson Immunocytometry System, Mountain View, CA) and then with rabbit fluorescein-conjugated anti-mouse IgG (1/50 dilution; DAKO).

**Results**

**Intact hamster tongue epithelium**

**Northern blot analysis.** Under stringent conditions, clear bands of a 2.5-kilobase pair (kb) with the Cx26 probe and a 3.1-kb with the Cx43 probe were detected in hamster tongue epithelium (Figure 1). These are in accordance with the reported transcript sizes for mouse Cx26 and Cx43 mRNAs (9). Western blot analysis. Alkali-treated tissue homogenates of hamster tongue epithelium were examined by Western blot analysis.
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Fig. 3. Double staining for Cx26 and Cx43 in hamster tongue epithelium using a double-exposure technique. (a) Phase-contrast image. (b) Same section as stained with anti-Cx26 antibody and anti-Cx43 antibody. Green signals represent Cx26 revealed with fluorescein-conjugated anti-rabbit antibody, whereas red signals represent Cx43 revealed with Texas Red-conjugated streptavidin. (c) Higher magnification view of hamster tongue epithelium. Bar: a = 20 µm; c = 60 µm.

analysis using the same anti-Cx43 and anti-Cx26 antibodies utilized for immunofluorescence. As positive controls, we used mouse heart and liver for Cx43 and Cx26, respectively. The anti-Cx43 antibody reacted with at least three bands, with molecular masses of ~42, 43 and 44 kDa. The anti-Cx26 antibody showed an ~24-kDa band and 34-kDa band in hamster tongue epithelium (Figure 2). No bands were found in the absence of the primary antibody under otherwise similar conditions (data not shown).

Immunofluorescence. Hamster tongue epithelium was thick enough that the different layers of stratified keratinocytes were easily recognizable (Figure 3a).

When the polyclonal antibody against Cx26 was used, there was macular staining on plasma membranes between keratinocytes in the granular layers and in the upper part of the squamous layers (Figure 3c). No fluorescent spots were found in basal keratinocytes with the anti-Cx26 antibody. In contrast, with the mouse monoclonal antibody against Cx43 there were macular fluorescent plaques on membranes between keratinocytes in the basal layer and in the lower part of the squamous layer. Cx43-positive spots were scarcely found in keratinocytes in the granular layer (Figure 3b). Under higher magnification, Cx26 and Cx43 were sometimes found to be co-localized at the same spots in the middle of squamous layers (Figure 3c). With the anti-Cx32 antibody, no specific staining was observed throughout the hamster tongue epithelium (data not shown). In the absence of the first antibody, only a little unspecific fluorescence was noted in the hamster tongue (data not shown).

In situ hybridization. Using an antisense probe for Cx26, positive signals were found in keratinocytes from basal layers to granular layers, mainly around the nucleus (Figure 4b). On the other hand, by use of the antisense probe for Cx43, positive signals were detected in keratinocytes in the basal and squamous layers, mainly around the nucleus, as well as in some stromal cells in the lamina propria (Figure 4c). The localization of Cx26 and Cx43 transcripts overlapped considerably, although Cx26 mRNA expression extended to higher layers than Cx43. No positive signals were observed using an antisense probe for Cx32 (Figure 4d) and sense probes for Cx26 or Cx43 (Figure 4e and f).

Wound healing

The light microscopical findings on wound-healing processes in the hamster tongue epithelium were in close agreement with those on wound healing in human skin (36). At 6 h, there was no obvious movement of epithelium into the wound. At 24 h, a tongue of epithelium started to invade the underlying connective tissue at each side of the wound. Between 24 and 48 h epithelial cells began to migrate from the wound edge toward the center of the wound. Epithelization of the wounds was complete by day 3. After wound closure, newly regenerated epithelium became highly stratified and thicker than the normal surrounding epithelium (day 5). The thickness of the tongue epithelium decreased to nearly normal by day 7 after wounding.

Using an antisense probe for Cx43 and Cx26, mRNA expression of Cx43 and Cx26 was maintained even at wound edges (Figure 5b and c). However, in situ hybridization revealed that mRNA expression of Cx43 and Cx26 was maintained even at wound edges (Figure 5b and c).

At 24 h after injury, immunofluorescence showed decreases in both Cx26 and Cx43 protein expression at wound edges (Figure 5b and c). However, in situ hybridization revealed that mRNA expression of Cx43 and Cx26 was maintained even at wound edges (Figure 6b and c). At 24 h after injury, expression of both Cx43 and Cx26 proteins was still low at the leading edges. However, the area in which Cx26 expression was low was limited to the front of the wound edge. Furthermore, in the epithelium near wound edges, expression of both proteins increased and overlapped considerably. Cx26-positive spots were found in keratinocytes only in the suprabasal layers but also in the basal layer, where Cx26 was not present in intact epithelium (Figure 5e). Similarly, Cx43-positive spots were localized in keratinocytes from basal to granular layers (Figure 5f). At a higher magnification, we found that both Cx4 proteins were frequently localized in the cytoplasm around nuclei in addition to the plasma membrane. In situ hybridization for Cx26 and Cx43 mRNAs showed strong positive signals in the cytoplasm.
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Fig. 4. In situ hybridization of Cx26, Cx43 and Cx32 mRNA in hamster tongue epithelium. (a) Hematoxylin and eosin staining. Positive signals were detected for Cx26 mRNA (b) and Cx43 mRNA (c). For Cx32 mRNA (d), the control Cx43 (e) and Cx26 (f) sense probes, no positive signals were observed. Bar = 10 μm.

of keratinocytes from the basal layer to the granular layer, indicating increases in both expression of connexins and overlapping (Figure 6e and f).

Three days after injury, Cx26 and Cx43 proteins were expressed strongly at wound edges and the nearby epithelium (Figure 5h and i). Co-localization of both Cx proteins to the same spots was frequently observed as at 24 h.

Five days after injury, although increased expression of Cx26 and Cx43 proteins continued, the expression of Cx26 protein appeared to be more localized in upper layers (Figure 5k), whereas that of Cx43 protein seemed to be more prominent in lower layers (Figure 5l). In other words, differential expression of Cx26 and Cx43 proteins seemed to begin to recover.

Seven days after injury, the differential expression of Cx26 and Cx43 proteins and localization on plasma membranes were completely recovered (data not shown).

Tongue carcinogenesis

Connexin expression in papillomas. The first tumor, tongue papilloma, appeared in the 8th week of the DMBA treatment. The incidence of papillomas increased progressively up to the 24th experimental week. Histopathology of hamster tongue papillomas induced with DMBA was almost the same as that of mouse skin papillomas induced by the DMBA/TPA protocol, i.e. the papillomas consisted of hyperplastic stratified squamous epithelium covered with cornified tissue, and different layers such as basal, squamous and granular layers could be recognized as in intact epithelium (Figure 7a). In papillomas, Cx32 protein was localized in keratinocytes not only in the basal and squamous layers but also often in the granular layer, where Cx43 was not present in intact tongue epithelium (Figure 7c). Similarly, Cx26 protein was found in keratinocytes in all layers (Figure 7b). The overlapping expression of Cx26 and Cx43 proteins was in agreement with that in mouse skin papillomas, which we have recently reported (27). In situ hybridization for Cx26 and Cx43 mRNAs showed that the expression of both mRNAs in papillomas increased compared with that in intact tongue epithelium and was found in all layers except for cornified layers (Figure 8b and c).

Connexin expression in squamous cell carcinomas. The first squamous cell carcinoma appeared at week 18 of the experiment. We analyzed squamous cell carcinomas, of which 10 were well differentiated, four were moderately differentiated and two were poorly differentiated as classified according to the differentiation phenotype.

Well-differentiated squamous cell carcinomas showed peripheral palisading of basal keratinocytes and the terminally differentiated (horny-like) structure called horny pearls (Figure 7d). In these carcinomas, Cx26 protein was expressed on the membranes of differentiated keratinocytes (Figure 7e), whereas Cx43 protein was localized on the membranes of tumor cells in a wide area (Figure 7f). The expression of Cx26 protein seemed to decrease in part compared with that in papillomas. In situ hybridization for Cx mRNAs showed that Cx43 mRNA was diffusely expressed in whole areas of carcinoma cells (Figure 8f), whereas Cx26 mRNA expression decreased compared with that in papillomas (Figure 8e).

Moderately differentiated squamous cell carcinomas contained few areas of terminal differentiation, but many of the tumor cells mimicked keratinocytes in basal and squamous layers (Figure 7g). Cx43 protein was expressed as macular spots on plasma membranes of tumor cells in a wide area (Figure 7i). On the other hand, the number of Cx26-positive spots strongly decreased in moderately differentiated squamous cell carcinomas compared with that in well-differentiated squamous cell carcinomas (Figure 7h).

Poorly differentiated squamous cell carcinomas showed more anaplastic nuclei, more mitosis and almost no keratiniz-
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Fig. 5. Immunofluorescence staining of Cx26 (b, e, h and k) and Cx43 (c, f, i and l) at 6 h after wounding (a, b and c), 24 h after wounding (d, e and f), 3 days after wounding (g, h and i) and 5 days after wounding (j, k and l). (a, d, g and j) Hematoxylin and eosin staining. (a) is a serial section of (b) and (c), both of which are from the same field, as are (d), (e) and (f). The same is true for (g), (h) and (i) as well as (j), (k) and (l). Bar = 10 µm.

In squamous cell carcinomas, we frequently found that Cx proteins were localized in cytoplasm, especially around nuclei, rather than on plasma membranes between cells (Figure 9c and d). The aberrant localization of Cx proteins was also observed during wound healing and in papillomas (data not shown).

Discussion

In the normal hamster tongue epithelium, we found that Cx43 protein was localized in keratinocytes in the basal layer and
in the lower part of the squamous layer. On the other hand, Cx26 protein was preferentially localized in the granular layer and in the upper part of the squamous layer. These data are consistent with those we reported for the mouse skin (9). Considering these data together, we may say that the modulation of Cx protein expression occurs during differentiation of keratinocytes in various squamous epithelia.

The mechanisms underlying normal keratinocyte differentiation involve the regulation of expression of a number of structural and non-structural protein genes (37). For example, in the basal layer, keratinocytes synthesize keratins K5 and K14, whereas in suprabasal cells, expression of K5 and K14 genes is repressed and synthesis of a new subset of differentiation-specific keratins, K1 and K10, is initiated and these become the quantitatively predominant proteins of differentiated keratinocytes. It has been shown that keratin expression is largely regulated at the transcriptional level (38). One might expect that the expression of Cx43 protein in the basal layer and that of Cx26 protein in the suprabasal layers would be regulated at the level of transcription, as in the case of keratin gene expression. However, we demonstrate here that in hamster oral epithelium the localization of Cx43 and Cx26 transcripts overlapped considerably, i.e. Cx43 transcripts were found in basal and squamous layers and Cx26 transcripts existed from basal to granular layers. These data suggest that the different localization of Cx43 and Cx26 proteins in squamous epithelium is largely regulated at post-transcriptional levels. A difference between transcript and protein distribution was reported for casein kinase 2 in the mouse skin during late embryogenesis by Mestres et al. (39). They found a high number of transcripts in the cells of the basal layer of the epidermis, but much less protein.

We show here that expression and localization of Cx proteins and transcripts were drastically changed in hamster tongue epithelium during wound healing, i.e. the expression of Cx43 and Cx26 proteins was reduced at the wound edge at 6–24 h after injury, but expression of both proteins increased in the epithelium near the wound edge at 24 h after injury. At 3–5 days after injury the expression increased at wound edges and the nearby epithelium, and then the expression and localization of both proteins recovered 7 days after injury. The increase in the protein expression was preceded by an increase in Cx transcripts. Wound healing of squamous epithelium is a complex process that involves many different cell types in migration, proliferation and differentiation, removal of damaged tissue, and production of extracellular matrices. There are only a few reports on changes in Cx expression and GJIC after experimental wounding. Pepper et al. (23) using an in vitro model in which a confluent monolayer of capillary endothelial cells was mechanically wounded, reported that the frequency and extent of GJIC were initially unaffected, but subsequently decreased and then markedly increased along the wound edge. The increased GJIC correlated with the presence of morphologically identifiable gap junctions between cells lining the wound edge at 24 h after wounding. When the wound had closed, the increased GJIC returned to the lower values observed in the non-wounded regions of the monolayer. They showed that when cell migration was prevented, the induction of wound-associated GJIC was also inhibited, suggesting that the increase in GJIC is dependent on cells migrating into the wound area. In a subsequent study, they revealed that the wound-induced increase in GJIC was accompanied by an increase in Cx43 protein and mRNA (24). Our present results showing that the decrease in Cx proteins and subsequent increase in the expression of Cx proteins and mRNAs occurred during wound healing of hamster tongue epithelium in vivo basically agree with those reported by Pepper et al. (23,24).
Recently, Goliger and Paul (26) reported that Cx expression wound edge and the wound periphery. Our present results agree with their findings in many respects. Cx26 and Cx43 were down-regulated in cells at the wound edge and at the leading edge of the regenerating epithelium. The expression of Cx26 was strongly up-regulated and extended into more layers in the epithelium near the wound edge and in the

Fig. 7. Immunofluorescence staining of Cx26 (b, e, h and k) and Cx43 (c, f, i and l) in a hamster tongue papilloma (a, b and c), well- (d, e and f), moderately- (g, h and i) and poorly-differentiated squamous cell carcinomas (j, k and l). (a, d, g and j) Hematoxylin and eosin staining. (a) is a serial section of (b) and (c), both of which are from the same field as are (d), (e) and (f). The same is true for (g), (h) and (i) as well as (j), (k) and (l). Bar = 20 µm.
Fig. 8. **In situ** hybridization of Cx26 (b and e) and Cx43 (c and f) mRNA in a hamster tongue papilloma (a, b and c) and well-differentiated squamous cell carcinoma (d, e and f). (a and d) Hematoxylin and eosin staining. (a), (b) and (c) are serial sections. These indicate the same field as do (d), (e) and (f). Bar = 10 µm.

Fig. 9. The aberrant localization of Cx26 (c) and Cx43 (d) in squamous cell carcinomas of hamster tongues. Cx26 and Cx43 proteins were observed in cytoplasm, particularly around nuclei. (a) and (b) Normal tongue epithelium. (a) Cx26; (b) Cx43. Bar = 50 µm.

Hyperproliferative epithelium. However, concerning Cx43 expression during wound healing, there is a discrepancy between our results and those of Goliger and Paul (26). We found increased expression of Cx43 in the epithelium near wound edges at 1–3 days after injury, whereas they reported that the Cx43 level was nearly the same in the hyperproliferative epidermis as in unwounded areas with regard to both intensity and location. We cannot specify the reason for the discrepancy, although differences in species and samples, i.e. hamster tongue and rat tail skin, might be a possible explanation.

Wound healing processes are mediated by a large number of growth factors and cytokines. Endogenous growth factors including the epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and transforming growth factor (TGF) beta families, are released at the wound site and presumed to be a necessary part of the natural wound healing machinery (40–45). On the other hand, GJIC and Cx expression are reported to be modulated by various growth factors. For example, basic FGF increased Cx43 mRNA and proteins and GJIC in microvascular endothelial cells (25) and cardiac fibroblasts (46). Furthermore, Pepper et al. (25) demonstrated that anti-bFGF antibodies inhibit the increase in GJIC and Cx43 expression observed after mechanically wounding a confluent monolayer of microvascular endothelial cells. It has also been reported that TGF-beta enhances the expression of Cx mRNA and proteins, and GJIC in normal rat kidney cells (47), C3H/10T1/2 cells (48) and human osteoblastic cells (49) *in vitro*. By contrast, EGF has been reported to inhibit GJIC (50–53). Madhukar et al. (50) reported that TGF-beta reduces GJIC in normal human epidermal keratinocytes *in vitro*. It is very likely that growth factors released at the wound site play important roles in the reduction of Cx proteins at the wound edge at early stages and subsequently increase as we have demonstrated in this study.

Several laboratories, including ours, have investigated sequential changes in the expression and localization of Cxs during multistage carcinogenesis using animal models, such as rat liver (15–22). There are only a few reports concerning such changes during carcinogenesis of squamous epithelium *in vivo* (27,28). Using multistage mouse skin carcinogenesis *in vivo*, we have recently shown that in papillomas no clear reduction of Cx43 and Cx26 is observed, that both proteins are frequently co-localized in the same gap junction plaques, and that in squamous cell carcinomas the expression of both proteins decreases and the expression of Cx26 is reduced as cancer cells become morphologically less differentiated (27).
Budunova et al. (28) have recently reported that in SENCAR mouse skin carcinogenesis, Cx43 and Cx26 are hyperexpressed in most papillomas, and immunostaining of squamous cell carcinomas revealed decreased Cx43 and Cx26 levels in 65% and 85% of cases, respectively.

In the present study we analyzed multistage hamster tongue carcinogenesis by the combination of immunofluorescence and in situ hybridization to examine the molecular mechanisms responsible for the changes in expression and localization of Cxs. We found that the expression of mRNAs and proteins of Cx43 and Cx26 increased and both proteins co-localized to the same plaques in papillomas. These data confirmed our previous results in papillomas formed during mouse skin carcinogenesis, and show that no clear reduction of the two Cx proteins occurred, and that Cx26 and Cx43 were frequently co-localized to the same plaques. The result obtained by in situ hybridization suggests that transcriptional upregulation and/or an increase in mRNA stability of Cx genes may be involved in the increase and overlapping expression of Cx43 and Cx26 proteins in papillomas.

In squamous cell carcinomas induced in hamster tongues, the expression of Cx26 protein was reduced as cancer cells became morphologically less differentiated, whereas that of Cx43 did not change. The present data obtained using hamster tongues are in good agreement with those we reported for squamous cell carcinomas formed during squamous cell mouse carcinogenesis (27), although the expression of Cx43 protein did not decrease clearly in hamster tongue carcinogenesis. Furthermore, we found that in squamous cell carcinomas Cx proteins were frequently localized in cytoplasm, especially around nuclei, rather than on plasma membranes between cells. The aberrant localization of Cx proteins is analogous to that of Cx32 protein reported in human (54) and rat liver cancers (22). These data suggest that GJIC is impaired in squamous cell carcinomas not only by decreased expression, but also by aberrant localization of Cx proteins. The mechanism for the intracytoplasmic localization of Cxs in tumors might be dysfunction of cell adhesion, for example, in the cadherin-catenin system, mutation of Cx genes or abnormal intracytoplasmic transport machinery. The data presented here show that quantitative and qualitative changes in Cx expression are associated with differentiation, migration and proliferation of keratinocytes in tongue squamous epithelium. Recent studies have revealed that each gap junction made of different Cxs has different channel properties, such as voltage dependence, unitary conductance and permeability, and that in certain combinations two Cxs can form heterotypic junctions and heteromeric hemichannels, with channel properties different from those in homotypic junctions (55). It has been hypothesized that different Cx channels have different permeabilities to second messenger molecules in signal transduction and that the differences may determine their specific roles in various biological function. Rapid progress in genetic approaches, such as production of Cx knockout mice (56), will certainly help us to further elucidate the role of GJIC in differentiation, migration and proliferation of keratinocytes in squamous epithelium.

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