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SHORT COMMUNICATION

In situ labeling of fragmented nuclear DNA in the desquamation of junctional and gingival epithelia in rats

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Introduction

Physiological cell death, or so-called programmed cell death, occurs via apoptosis in embryonic and fetal tissues. Apoptosis has been distinguished from necrosis ever since Kerr, *et al.*[1] described in detail the ultrastructural changes characteristic of dying cells, i.e. nuclear condensation and cytoplasmic shrinkage, with a release of apoptotic bodies[2]. Since apoptosis can be characterized biochemically by DNA fragmentation, it can also be detected by a DNA ladder in agarose gel electrophoresis. McCall and Cohen[3] performed an *in vitro* study which showed that cell death in terminally differentiating keratinocytes was programmed cell death with DNA fragmentation by DNA gel electrophoresis. In addition, Gavrieli, *et al.*[4] reported a novel technique which incorporates specific labeling of nuclear DNA fragmentation, "DNA nick end labeling", to identify DNA fragmentation in paraffinized tissue sections. Recently, we used this DNA nick end labeling method to show apoptosis in normal oral epithelium and squamous cell carcinoma in humans[5].

In this study, we demonstrated programmed cell death by using DNA nick end labeling at terminal differentiation, i.e. desquamation, in situ in junctional epithelium and gingival epithelia in adult and fetus, and examined the rate of desquamation in adult and developing gingival epithelia in rats.

Materials and Methods

Twenty-day in utero and 2-month-old adult Sprague-Dawley rats were used in this study. Maxillaries were removed from the animals, fixed in 10 % buffered formalin, and demineralized in 10 % EDTA. The tissues were embedded in paraffin and sectioned at 4 μm.

DNA nick end labeling was performed according to Gavrieli[4], *et al.* In brief, the paraffinized sections were treated with 10 μg/ml proteinase K (Boehringer Mannheim, Germany) in 10 mM Tris-HCl buffer, pH 7.4 at room temperature (RT) for 20 min, and then incubated with 2 % H_2O_2 for 7 min at RT to block endogenous peroxidase activity. Each section was rinsed well in distilled water (DW) and immersed in TdT buffer, pH 7.2 (0.5 M potassium cacodylate, 10 mM $CoCl_2$, 1 mM DTT) (Gibco Brl., USA) for a couple of minutes. Samples were then incubated with TdT (terminal deoxynucleotidyl transferase, 0.3 equivalent unit/μl) (Gibco Brl., USA) containing biotin-16-UTP (biotin-16-2'-deoxyuridine-5'-triphosphate, 0.04 nmol/μl) (Boehringer Mannheim, Germany) and TdT buffer diluted 5-fold in DW at 37°C for 90 min. Specimens were immersed in TB buffer (3 mM sodium citrate, 30 mM sodium chloride) for 30 min at RT, washed with DW, and then washed with

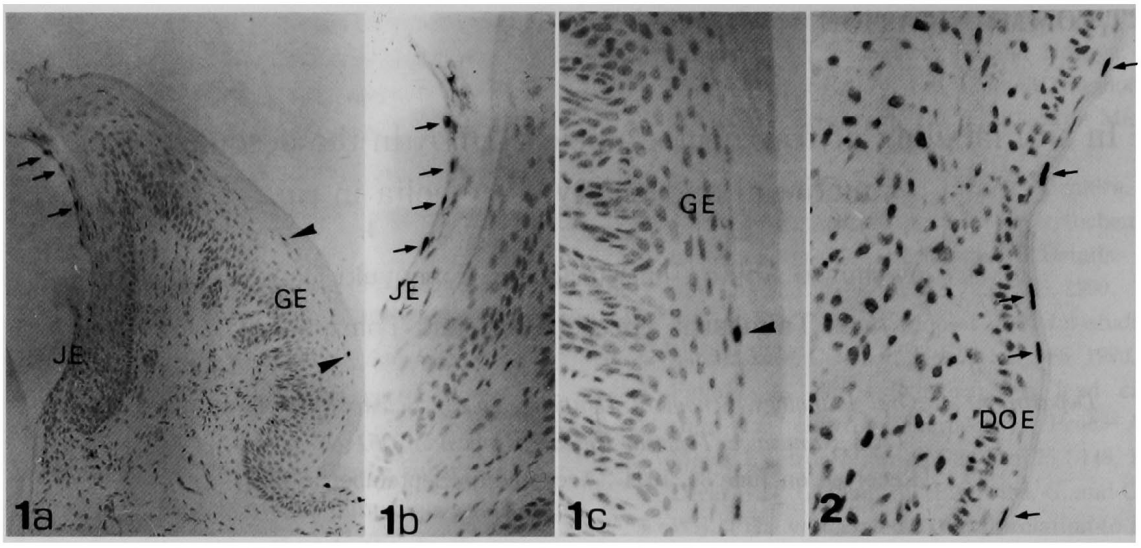

Fig. 1 Signals of DNA fragmentation in adult junctional and gingival epithelia.
1a : Positive staining was found at the sulcus of junctional epithelium (JE, arrows) and at the part of the subcornified layer in the gingival epithelium (GE, arrowheads). 1b : Higher magnification of JE ; 1c : Higher magnification of GE. Signals are on the nuclei.

Fig. 2 Signals of DNA fragmentation in developing oral epithelium. The developing oral epithelium (DOE) is obviously thinner and shows a more positively stained nuclei (arrows) than adult GE (Fig 1c).

PBS.

Peroxidase-conjugated streptavidin (streptavidin-biotin kit, Histofine, Nichirei, Japan) was reacted with the biotin-16-UTP, and finally the reactions were visualized by DAB (0.05 % diaminobenzidine and 0.005 % H_2O_2 in 0.05 Tris-HCl buffer, pH 7.6). The rate of desquamation in these epithelia was expressed as the number of nick-end positive nuclei/number of nuclei in the basal cell layer in the epithelia. The number of nick-end positive nuclei within 125 μm was counted in triplicate in ten fields in each of eight samples magnified at $\times$320 under a light microscope. Statistical comparisons were performed using Student's t-test.

Results and Discussion

In adult tissues, some nuclei in the area of the gingival sulcus in junctional epithelium and at the subcornified layer in oral epithelium displayed positive nick end labeling (Fig. 1 a, b, c). Positive nuclei were also observed at the cornified layer with parakeratosis in developing oral epithelium (Fig. 2). Although all of the cells in the junctional epithelium are believed to exfoliate at the relatively narrow free surface of the junctional epithelium[6], no previous report has shown this phenomenon in situ. Thus, our present results confirm previous reports regarding the location of desquamation in the junctional epithelium. Since the desquamation of junctional epithelium is also a type of physiological cell death, it may be apoptotic cell death, even though DNA nick end labeling shows random DNA fragmentation rather than DNA fragmentation specific for apoptosis. Löe and Karring[7] introduced the mitotic index to express mitotic activity as the number of dividing cells per square millimeter. Subsequently, Skougaard[8] suggested that the main advantage of this index would be its ability to describe the rate of desquamation, since, under normal conditions, desquamation at the surface is matched by a similar rate of cell renewal at the basement membrane to maintain a fairly consistent

Table 1 The rate of desquamation in adult and developing oral epithelia.

Adult oral epithelium	0.0151±0.0139
Developing oral epithelium	0.0624±0.0303

Significant difference ($p<0.001$)
Desquamation index=the number of nick-end positive nuclei/the number of nuclei in the basal cell layer.

thickness of stratified squamous epithelium. We estimated the rate of desquamation in epithelia by counting positive nuclei by nick end labeling. This index might give a more precise estimation of the rate of desquamation, since positive labeling reflects the actual state of exfoliation. As shown in Table 1, the desquamation index in developing oral epithelium was significantly higher than that in adult oral epithelium. Although the desquamation index in junctional epithelium could not be estimated because the border between basal cells of junctional epithelium and the cells of gingival sulcus epithelium was unclear, it was obvious that there was more nick end labeling in junctional epithelium. These results are consistent with previous findings that the turnover time for junctional epithelium is much faster than that for gingival epithelium[6,8]. Listgarten[6] reported that a thicker epithelium would require a longer turnover time than a thinner epithelium. Our results suggest that the rate of desquamation reflects the turnover time, and that desquamation by apoptosis plays an important role in the maintenance of junctional and gingival epithelia, including developing epithelium.

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