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Flow Cytometric DNA Content Analysis of Ultraviolet Light-induced Squamous Cell Carcinomas: A Comparative Study of Squamous Cell Carcinomas of the Lip and Those Arising from Other Sites of Sun-damaged Skin

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The development of squamous cell carcinoma (SCC) of the lip is considered to be mainly related to excessive sun exposure. However, its higher metastatic rate is distinct from that of SCCs arising from other sites of sun-damaged skin. Flow cytometric DNA content analysis, using paraffin-embedded specimens, was performed on 15 SCCs of the lip and 32 SCCs arising from other sites of sun-damaged skin. A significantly lower incidence of DNA-aneuploidy was observed in SCCs of the lip (2/15) than in those of sun-damaged skin (15/32) ($p<0.05$). The mean age of patients with SCC of the lip (66.7±11.6 years; mean±SD) was significantly lower than that of the other patients (78.1±11.1 years) ($p<0.01$). There was no significant difference between the mean diameter size of tumors on the lip (19.5±5.7 mm) and that of tumors on other sites of sun-damaged skin (30.7±20.5 mm). These results suggest that additional carcinogenic factors besides ultraviolet light may be involved in the development of SCC of the lip.
Key words: DNA-ploidy; flow cytometry; skin cancer.

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Squamous cell carcinoma (SCC) of the skin usually arises from various preceding lesions, such as sun-damaged skin, burn scar and chronic radiodermatitis. Among these preceding skin conditions, it arises most commonly in sun-damaged skin. SCC of the lip, which almost always involves the vermilion of the lower lip, is also considered in most cases to be related to sun exposure. However, its higher metastatic rate is distinct from that of SCCs arising from other types of sun-damaged skin (1–6). DNA-aneuploidy as a marker for malignant tumor cells has been investigated upon various skin tumors (7–10). Concerning SCCs of the skin, DNA-aneuploidy has been detected in about 20% of the tumors (11, 12). However, the relationship between DNA-ploidy and the preceding lesions has been poorly examined. We have already reported that 46.2% of SCCs arising from sun-damaged skin showed DNA-aneuploidy, while none of the SCCs from burn scars did (13). The present flow cytometric analysis was aimed at clarifying the differences of DNA-ploidy between SCCs of the lip and of other sites of sun-damaged skin, both of which have been considered to be induced by ultraviolet (UV) light exposure.

MATERIAL AND METHODS

Subjects

Forty-seven primary SCCs arising from a sun-damaged region, including the lip, were used for this study. The materials were taken from surgically resected specimens from 46 patients (31 males and 15 females), who visited our clinic between 1981 and 1994. SCCs related with obvious preceding skin disorders other than UV-related lesions, such as burn scar and discoid lupus erythematosus, were excluded from this study, even if they had developed on the lip or sun-damaged skin. The materials consisted of 15 SCCs developed on the lip and 32 on other sites of sun-damaged skin. All SCCs of the lip had developed on the vermilion border of the lower lip. The other 32 SCCs on sun-damaged skin arose in 31 elderly patients. In the cases with a large lesion from which several blocks were taken, the most tumor cell-rich blocks were selected for the study. Histopathological grading, using the system proposed by Broders (14), was applied to all specimens blindly to the DNA-ploidy and the location of the lesion.

Sample preparation

All specimens were fixed in 10% formalin and embedded in paraffin wax with a routine method. Preparation for flow cytometry was made with a modified method of Hedley et al. (15). Six or seven leaves of a 30-μm-thick section from a specimen were dewaxed with three changes of xylene for 10 min each at room temperature and rehydrated with 100, 95, and 70% ethanol for 10 min each time at room temperature. Then the sections were washed in distilled water and digested with 0.5% pepsin (Sigma, St Louis, MO, U.S.A.) in 0.9% NaCl at pH 1.5 for 30 min at 37°C with intermittent vortex mixing. The cells were stained with 50 μg/ml of propidium iodide (Sigma) in calcium- and magnesium-free, phosphate-buffered saline, containing 1 mg/ml RNase A type I-A (Sigma), for 30 min at room temperature, then filtered through nylon mesh to remove aggregates. At the same time, 5-μm-thick sections for hematoxylin and eosin staining were prepared, to confirm the histological finding of the sections used for DNA analysis.

Flow cytometry

The relative DNA content of at least 10,000 nuclei per sample was measured by flow cytometry (Fluorescence Activated Cell Analyzer-FACScan, Becton Dickinson, CA, U.S.A.) with an argon-ion laser operating 15 mW at 488 nm for excitation. All samples contained inflammatory and stromal cells, which served as an internal diploid standard (9). A sample showing a single peak (G_0/G_1 phase) often followed by a minor peak (G_2+M phase) was classified as diploid, while a sample with another G_0/G_1 peak containing 10% or more of total nuclei was classified as aneuploid. Statistical analysis was performed by an unpaired *t*-test or chi-square test. A *p* value less than 0.05 was considered as significant.

RESULTS

Each sample examined had a definite diploid G_0/G_1 peak (Fig. 1a). Thirty out of 47 samples showed the diploid pattern alone, while 17 samples additionally exhibited one or more minor G_0/G_1 peaks (Fig. 1b). Table I summarizes the relation between the DNA-ploidy and the location of SCC. DNA-aneuploidy was observed in only 2 (13.3%) out of 15 SCCs on the lip, but in 15 (46.9%) out of 32 SCCs on sun-damaged skin. The incidence of DNA-aneuploidy in SCCs of the lip

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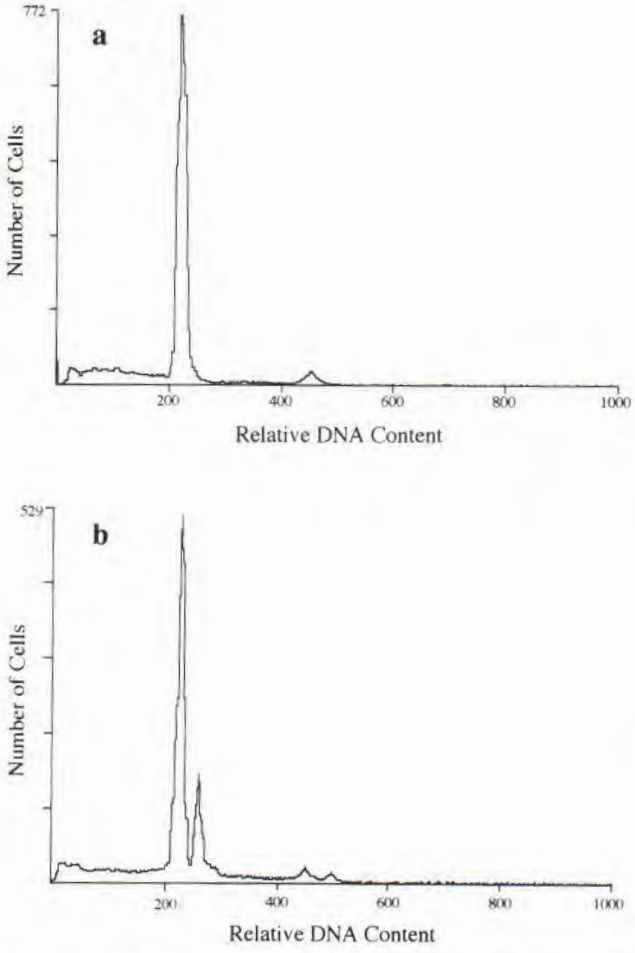

Fig. 1. (*a*) DNA histogram of SCC, showing diploid pattern. (*b*) DNA histogram of SCC, showing aneuploid pattern.

Table I. *DNA-ploidy of SCCs arising on the lip and other sites of sun-damaged skin*

Location of tumors	Number of specimens	DNA-ploidy		
		Diploid	Aneuploid	
Lip	15	13	2	$p<0.05$
Sun-damaged skin	32	17	15	

was significantly lower than that in SCCs of sun-damaged skin ($p<0.05$; chi-square test). With regard to the age distribution of the patients, SCC of the lip most frequently arose in the seventh decades, while the number of patients with SCC from sun-damaged skin increased with age (Table II). The mean age of the patients with SCC of the lip (66.7 ± 11.6 years; mean $\pm$ SD) was significantly lower than that of the patients with SCC on sun-damaged skin (78.1 ± 11.1 years) ($p<0.01$; unpaired *t*-test).

The size of 46 tumors and their relation to DNA-ploidy are shown in Table II. Only one tumor, which developed on the lip, was eliminated from the table for the reason that its precise size had not been recorded. Tumors with a size exceeding 40 mm were only found in the sun-damaged skin group, and the mean length of the largest diameter of tumors arising on the lip (19.5 ± 5.7 mm; mean $\pm$ SD) was smaller than that of tumors developed on sun-damaged skin (30.7 ± 20.5 mm). However, there was no significant difference between them. The incidence of DNA-aneuploidy was higher in the tumors exceeding 50 mm. However, aneuploid tumors were also frequently found in the smaller size group of 10–19 mm and 20–29 mm. The incidence of the smoking habits between the two groups of SCC of the lip and of sun-damaged skin is indicated in Table II. Eight (53.3%) of 15 patients with SCC of the lip had smoking habits, while 14 (45.2%) of 31 patients with SCC of sun-damaged skin did. The higher incidence of smoking habits in the group with SCC of the lip failed, however, to reach statistical significance.

We observed a tendency that the incidence of DNA-aneuploidy was higher as the histological grade was advanced (Table III). The incidence of DNA-aneuploidy in SCCs of the lip was always lower than that of sun-damaged skin in each histological grade, although statistically not significant.

DISCUSSION

SCC develops most commonly in sun-damaged regions, including the lip. SCC of the lip almost exclusively arises in the vermilion of the lip, often accompanying solar cheilitis, and is considered to be related to actinic damage of the mucocutaneous region. In primary cutaneous SCC, the risk of metastatic spread varies according to the preceding lesion associated with SCC (16). Actinically induced SCC has been regarded as a low-grade malignancy, very rarely giving rise to metastasis. The metastatic rate of 0.5% to 5.2% for patients with SCC arising from sun-damaged skin has been reported (6, 17, 18). However, SCC of the lip, which is also in most cases induced by sun-exposure, has a much higher incidence of metastasis, about 14% (6). The reason why the metastatic rate of SCC is different between the lip and other sun-exposed skin has not been clarified up to date. In the present study, SCCs of the lip revealed a significantly lower incidence of DNA-aneuploidy as compared with those of sun-damaged skin. Even in the same histological grade, SCCs of the lip showed a lower frequency of DNA-aneuploidy than those of sun-damaged skin, although statistically not significant. Furthermore, SCCs of the lip developed in significantly younger patients. In SCCs from sun-damaged skin, patients over 70 years dominated (more than 80%), while 60% of the patients with SCC of the lip were under 69 years. The earlier development of tumors on the lip would be partly explained by a smaller amount of melanin in the epithelium of the mucocutaneous region, which results in severe UV damage to the epidermis. However, this cannot explain the lower frequency of DNA-aneuploidy of SCCs on the lip than of those on sun-damaged skin. We have already reported that the incidence of DNA-aneuploidy differs depending on the preceding lesions (13). In the present studies, we demonstrated that there was a disparity regarding DNA-aneuploidy and the age of the patients between SCCs of the lip and those of sun-damaged skin. These results suggest that additional carcinogenic factors besides UV light may be involved in the development of SCC of the lip, although we failed to demonstrate the influence of smoking habits on the development of SCCs of the lip. In fact, recent studies have revealed that H-ras oncogene mutation, which has been proved to be frequently detected in human oral SCC associated with tobacco chewing (19) and in murine oral SCC induced by a chemical carcinogen (20), is also frequently found in SCC of the lip vermilion (21).

These reports, together with the present investigations,

Table II. *Comparison of clinical data of SCCs of the lip and sun-damaged skin*

		Age distribution					
Age in decades		40–49	50–59	60–69	70–79	80–	
No. of patients	Lip	1	2	6	4	2	
	Sun-damaged skin	0	3	3	12	13	
		Size of tumours					
Largest diameter (mm)		0–9	10–19	20–29	30–39	40–49	50–
No. of tumors	Lip	1	7	5	1	0	0
	Sun-damaged skin	0	8	13	4	1	6
DNA-ploidy (aneuploid/diploid)		0/1	4/11	8/10	1/4	0/1	4/2
		Smoking habits					
		smoking habits (+)		smoking habits (−)			
No. of patients	Lip	8		7			
	Sun-damaged skin	14		17			

Table III. *DNA-ploidy and histological grading in 47 SCCs*

Grade	Lip		Sun-damaged skin	
	Diploid	Aneuploid	Diploid	Aneuploid
1	10	1	13	6
2	2	1	3	5
3	1	0	1	3
4	0	0	0	1

suggest that SCCs of the lip may differ from those of other sites of sun-damaged skin in their carcinogenesis, and that some chemical carcinogens besides UV light may be partly involved in the development of SCC of the lip.

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