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Comparative Nuclear Morphometric Analysis of Aggressive and Non-aggressive Squamous Cell Carcinomas of the Skin

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Squamous cell carcinomas (SCC) are the most frequent tumours complicating organ transplantation. Whereas most SCC can be successfully treated with conventional surgery, other lesions show an aggressive course with recurrence and metastases. We assessed the value of nuclear morphometry in detecting tumours with an ab initio potential for aggressive course. Nuclear perimeter, area, feret X and feret Y were calculated semi-automatically on an image analyzer on histological sections of 15 non-aggressive, 15 aggressive and 6 recurrent SCC developed in seven organ graft recipients. We found statistically significant differences for all the parameters studied between recurrent and initially aggressive SCC, and, to a lesser extent, between non-aggressive and aggressive SCC. These results suggest that some SCC have ab initio a potential for more aggressive evolution; morphometry can be a useful adjunct in order to better study these lesions. ***Key words: nucleus; morphometry; organ transplantation.***

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Squamous cell carcinomas (SCC) of the skin are among the most frequent human malignancies in the white population, accounting for about 20% of cutaneous malignancies (1, 2). They are usually amenable to definite cure after conservative treatment (simple surgical excision); however, they occasionally have a more severe course, giving rise to multiple local recurrences and regional lymph node or visceral metastases, which may cause death. This is especially true in immunocompromised hosts (namely organ transplant recipients (OTR)), in which cutaneous SCC represent the most common malignancy (3–5). In view of the potentially life-threatening course of some SCC, their early recognition becomes necessary in order to proceed to an adequate treatment (larger surgical excision) and to a careful follow-up. Previous studies have suggested that nuclear morphometric features may correlate with the outcome of malignant tumours, such as cutaneous malignant melanoma (6–10), basal cell carcinoma (11), breast (12–16), renal (17) and laryngeal (18) carcinomas. To the best of our knowledge, no detailed morphometric study has ever been performed on cutaneous SCC. The aim of the present study was to assess the nuclear morphometric features of aggressive in comparison with non-aggressive SCC, in order to evaluate whether this method could be valuable for the early recognition of aggressive tumours. We chose to study SCC developing in OTR since these patients develop multiple SCC, with some lesions being aggressive and others non-aggressive; by studying tumours developing in the same patients, the influence of extrinsic factors (age, skin type, sun exposure, immunosuppressive treatment, etc) would be minimized, providing the possibility to detect the intrinsically aggressive features of each lesion.

MATERIAL AND METHODS

Patients

During the last 6 years, over 1,000 OTR were examined in our department for various dermatologic problems. Among 450 of them presenting proliferative (pre)malignant cutaneous lesions, 50 patients had SCC. Seven of them (5 kidney and 2 heart transplant recipients) developed multiple SCC (a total of 27). All patients were white men; the mean age was 51 ± 8 years. At least one SCC in each patient showed an aggressive course, i.e. was complicated by multiple local recurrences (6 cases) or lymph node metastases (one case), developed between 2 months and 3 years after the initial excision; a fatal outcome was noted in 3 patients. All patients had light-coloured skin and eyes and had experienced a heavy cumulative sun exposure.

Tumour specimens

Fifteen histologically confirmed SCC that were known thanks to the follow-up to exhibit an aggressive evolution (local recurrence(s) and/or regional metastases) were selected. Specimens of the initial as well as of the recurrent tumour (available for six of these lesions) were studied. A control group of fifteen SCC was also examined. These were non-aggressive SCC that had been been excised from the same patients at about the same period of time and from an equivalent location as the aggressive tumours. These SCC had been successfully treated by simple surgical excision. All specimens had been collected in our department over the past 6 years, formalin-fixed and paraffin-embedded. The diagnosis had been made after examination of haematoxylin-eosin-stained sections.

Morphometric study

The measurements were performed on 4 μm-thick haematoxylin-eosin-stained sections using a semi-automatic image analyzer (Videoplan, Kontron, Münich), coupled to a Zeiss light-microscope. An oil immersion objective (X100) was used (final magnification 1,000X). Measurements were made at high magnification, as previously recommended, so as to render results more reproducible (19), and by the same observer (DN) in order to avoid interobserver variations. A representative area of each tumour was chosen at scanning magnification, corresponding usually to the deepest part of each SCC; 300 nuclei located at the periphery of tumour masses were analyzed for each lesion. The following parameters were calculated: nuclear surface, nuclear perimeter, feret X (projection in X-direction) and feret Y (projection in Y-direction).

Statistical analysis

This was made by the non-parametric Mann & Whitney U-test.

RESULTS

All aggressive and non-aggressive SCC were located on the cephalic extremity, with few exceptions (forearm, shoulder, chest). The initial aggressive SCC were thick, infiltrating the subcutaneous adipose tissue and in 2 cases also the underlying cartilage and muscles. In all patients the recurrent SCC also showed invasion of the underlying tissues (cartilage and muscles and occasionally of bone and nerves). Non-aggressive SCC showed variable thickness.

The results of the morphometric analysis are summarized

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Table I. *Nuclear morphometric parameters of non-aggressive, aggressive and recurrent squamous cell carcinoma (SCC)*

Parameter	Non-aggressive SCC	Aggressive SCC	Recurrent SCC
Area*	54.9±15.0	45.8±11.8 (A)	61.3±10.2 (B)
Perimeter**	29.1±3.7 (C)	25.9±3.1 (D)	29.9±2.7 (E)
Feret X***	8.9±1.4	8.0±1.0 (F)	9.0±1.1 (G)
Feret Y****	8.8±1.1 (H)	7.9±1.0 (I)	9.6±0.5 (J)

Significant p values (Mann & Whitney U-test):
A vs B 0.01
C vs D <0.05
D vs E <0.05
F vs G <0.05
H vs I <0.05
H vs J <0.05
I vs J <0.01

* Mean value (±SD) expressed in μm^2
** Mean value (±SD) expressed in μm
*** Mean value (±SD) of projection in x-direction
**** Mean value (±SD) of projection in y-direction.

in Table I. Statistically significant differences were found for the following parameters: a) as compared with non-aggressive SCC, aggressive SCC had lower nuclear perimeters and lower feret Y values ($p<0.05$); b) as compared with non-aggressive SCC, recurrent SCC had higher feret Y values ($p<0.05$); c) as compared with (initial lesions of) aggressive SCC, recurrent SCC had higher mean values of all the parameters studied, i.e. nuclear area, perimeter, feret X and feret Y ($p=0.01$, <0.05, <0.05 and <0.01, respectively).

DISCUSSION

The course and prognosis of SCC depends on several factors, such as age, location of the lesion and immune status of the patient (1). In OTR additional factors influencing prognosis include time after transplantation, intensity of immunosuppressive treatment and possibly also the nature of the graft (5). However, there is evidence that some SCC are aggressive ab initio; this is upheld by our clinical observation that the same patient may at some time develop simultaneously both aggressive and non-aggressive SCC. The early recognition of aggressive SCC is important, because of the need of adequate treatment (wide surgical excision, tapering of the immunosuppressive treatment, addition of retinoids) and close follow-up (20). Up till now some histological parameters of SCC have been claimed to correlate with aggressivity and unfavourable prognosis. These include density of the inflammatory peritumoral infiltrate, tumour size and thickness, depth of invasion and perineural invasion (21, 22). As far as we know, nuclear features of cutaneous SCC have not been, until now, investigated in detail.

In our study, evaluation of the nuclear parameters measured showed differences between the three groups of SCC (non-aggressive, aggressive and recurrent) (Table I), and some of these proved statistically significant. In comparison with non-aggressive SCC, aggressive lesions had lower values of nuclear perimeter, reflecting a smaller nuclear size. This trend was also noted for nuclear area, even though this difference did not prove statistically significant (possibly due to a higher degree of variability in nuclear area as compared to perimeter). On the other hand, recurrent tumours had higher perimeter values as compared with both aggressive and non-aggressive SCC. This finding is in keeping with previous results concerning metastasizing melanomas (6–8, 19) and laryngeal carcinomas (14). Recurrent tumours were also found to have the smallest standard deviations in the features studied, suggesting that their nuclei were more uniform in size and shape. This finding is in keeping with results obtained on primary and metastatic breast carcinomas (13). Nuclei of recurrent tumours had similar feret X and feret Y values, characteristic of spherical nuclei. This is in keeping with previous reports suggesting an association of predominantly round nuclei with poorer prognosis (9).

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