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Predictive variables for the biological behaviour of basal cell carcinoma of the face: relevance of morphometry of the nuclei

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SUMMARY. **We did a morphometric analysis of 130 histological sections of basal cell carcinoma (BCC) of the face to find out whether morphometric variables in the structure of the nuclei of BCC cells could serve as predictors of the biological behaviour. We considered the following variables: maximum and minimum diameters, perimeter, nuclear area and five form factors that characterise and quantify the shape of a structure (axis ratio, shape factor, nuclear contour index, nuclear roundness and circumference ratio). We did a statistical analysis of primary and recurring tumours and four histology-based groups (multifocal superficial BCCs, nodular BCCs, sclerosing BCCs and miscellaneous forms) using a two-sided *t* test for independent samples. Multifocal superficial BCCs showed significantly smaller values for the directly measured variables (maximum and minimum diameters, perimeter and nuclear area). Morphometry could not distinguish between primary and recurring tumours.**

Keywords: **Basal cell carcinoma; Biological behaviour; Recurrence; Morphometric analysis; Nuclei**

INTRODUCTION

Basal cell carcinoma (BCC) of the skin is the most common type of human neoplasm and accounts for 50–75% of all skin tumours.[1] After complete resection of a BCC in the head and neck area, the prognosis is generally good.[2] Reported recurrence rates vary between 0.5 and 14%,[3] and metastases are extremely rare.[4–6] Despite numerous attempts at histological and clinical classification, it has not been possible to deduce the biological aggressiveness of BCCs from their histological characteristics. Several factors contribute to this, including the fact that various degrees of differentiation can be found within a tumour. The introduction of morphometry in histopathology has allowed the measurement of structural and mophological characteristics of cells and identification of variations from the norm. The purpose of the present study was to explore whether there is a correlation between morphological characteristics of the nuclei of BCC cells and the aggressiveness and recurrence tendency on the one hand, and clinical morphological types on the other.

PATIENTS AND METHODS

Patients

A total of 130 histological sections of BCCs, which were excised from 126 patients between the years 1986 and 1997, were studied retrospectively. There were 49 women and 77 men. The mean age at the time of operation was 65 years (range 18–101). All tumours were resected with a margin of 1–2 mm using a two-stage operation technique, controlled by microscopy as described previously.[7] When histological examination had confirmed that the margins were free of tumour, the defect was repaired secondarily. During the histological processing period of 2–3 days, the resection area was covered temporarily with a sterile dressing. We excluded the samples from patients at high risk of skin tumours and patients who had had radiotherapy.

Methods

With a standard colour video CCD-camera (Sony 77 CE, Sony Germany GmbH, Cologne, Germany), mounted on a microscope (Olympus BHS, Olympus optical Co. (Europe) GmbH, Hamburg, Germany), the histological preparations were photographed on a monitor for analysis. A 100-fold objective with immersion oil was used and the device was adjusted with an object-micrometer. This enabled us to express the distances measured directly in micrometers. The measurements were made on 4 μm thick, haematoxylin–eosin stained preparations. Using a PC-based image processing system (Bio Scan OPTIMAS®, Bio Scan Inc., Seattle, WA, USA) and a framegrammercard, the borders of the nuclei were

Table 1 Form factors that describe the shape of a structure in relation to a full circle

Axis ratio	area/(1/4π × length × width)
Shape factor (Saltykov)	4π × area/(circumference)^2
Nuclear contour index	circumference/$\sqrt{}$area
Nuclear roundness	circumference/(2 × $\sqrt{}$(π × area))
Circumference ratio	(circumference)^2/4π × area

Form factors can be expected to be relevant when the nuclear borders are irregular or the nuclear shapes differ. This may be an indication of potentially aggressive behaviour.

circumscribed and digitised with the PC. On each of the 130 slides, 100 nuclei were selected, so that 13 000 nuclei were measured for length (largest diameter of the nucleus), width (smallest diameter of the nucleus), circumference and area.

From these data the following five form factors were derived: axis ratio, shape factor, nuclear contour index, nuclear roundness and circumference ratio (Table 1).

These form factors – in contrast to the linear morphometry measurements, such as diameter, circumference and area – are dimensionless and allow the quantitative acquisition of the shape of a structure. All form factors selected in this study aimed to measure the deviation of a full circle of a nucleus section. Form factors can be expected to be relevant when the nuclear borders are irregular or when there are differences in the nuclear shape. Our aim was to quantify the morphological changes of the nucleus as an indication of potential aggressive behaviour. Details about the selection of the single form factors are described elsewhere.[8–10]

Firstly, we evaluated the morphological differences between non-recurring and recurring tumours in the 130 cases. Then, we also defined four groups according to the International histological classification of tumours of the WHO:[11] multifocal superficial BCCs including superficial multicentric, nodular BCCs including solid and adenoid cystic, sclerosing BCCs including morpheic, and miscellaneous forms.

Statistical tests were done with Systat® 5.2 for Macintosh. After verifying a normal distribution, the means of the variables for the specific groups were calculated and compared with the two-sided Student's *t* test. Probabilities of less than 0.05 were accepted as significant.

RESULTS

Of the 130 tumours examined, 95 were primary (no history of a former BCC) and 35 were recurrent tumours at the same location. In 27 of the 35 recurrent tumours, the primary tumour had been excised elsewhere, so that the histological sections were not available for comparison. There were only four cases of primary and recurrent tumours in the same patient, which entered the total collective.

For the primary and recurrent groups the means (SD) of the directly measured variables and the derived form factors are shown in Table 2. There were no significant differences between these groups, either in the measurements or in the form factors.

The original pathological findings were then generated within the four histological groups to find out whether histological types show morphological subsumable characteristics for differentiation.

In the four histological groups the means (SD) of the directly measured variables and the calculated form factors are shown in Table 3. There is a considerable difference in the direct measurements between the group of multifocal superficial BCCs including superficial

Table 2 Measurements and calculated form factors of primary and recurrent tumours

	Primary (n = 95)	Recurrent (n = 35)
Length	8.29 (0.95)	8.36 (0.96)
Width	5.39 (0.77)	5.44 (0.82)
Circumference	22.47 (2.60)	22.75 (2.62)
Area	34.42 (8.29)	35.08 (8.89)
Axis ratio	0.96 (0.01)	0.96 (0.01)
Shape factor	0.82 (0.12)	0.83 (0.05)
Nuclear contour index	3.90 (0.11)	3.92 (0.12)
Nuclear roundness	1.10 (0.03)	1.11 (0.04)
Circumference ratio	1.21 (0.07)	1.22 (0.08)

Numeric values are mean (SD) in μm or μm^2, respectively, form factors are dimensionless.

Table 3 Measurements and calculated form factors of four histological types of BCC

	Multifocal (n = 4)	Nodular (n = 107)	Sclerosing (n = 15)	Miscellaneous (n = 4)
Length	7.00 (0.61)	8.34 (0.90)	8.46 (1.24)	8.15 (0.60)
Width	4.62 (0.56)	5.43 (0.77)	5.43 (0.90)	5.36 (0.46)
Circumference	18.92 (1.59)	22.62 (2.51)	23.03 (3.07)	22.28 (1.38)
Area	24.76 (4.81)	34.90 (8.18)	35.33 (10.40)	33.55 (5.29)
Axis ratio	0.96 (0.08)	0.96 (0.01)	0.96 (0.02)	0.96 (0.09)
Shape factor	0.85 (0.03)	0.82 (0.11)	0.81 (0.06)	0.83 (0.05)
Nuclear contour index	3.86 (0.07)	3.90 (0.11)	3.96 (0.15)	3.91 (0.12)
Nuclear roundness	1.09 (0.02)	1.10 (0.03)	1.12 (0.04)	1.10 (0.04)
Circumference ratio	1.19 (0.04)	1.21 (0.07)	1.25 (0.10)	1.22 (0.08)

Numeric values are mean (SD) in μm or μm^2, respectively, form factors are dimensionless.

Table 4 Results of the *t*-tests multifocal compared with (a) nodular, (b) sclerosing and (c) miscellaneous forms

	Multifocal ($n = 4$)	Nodular ($n = 107$)	*P* value
Length	7.00 (0.61)	8.34 (0.90)	0.004
Width	4.62 (0.56)	5.43 (0.77)	0.04
Circumference	18.92 (1.59)	22.68 (2.51)	0.004
Area	24.76 (4.81)	34.90 (8.18)	0.02
	Multifocal ($n = 4$)	Sclerosing ($n = 15$)	*P* value
Length	7.00 (0.61)	8.46 (1.24)	0.04
Width	4.62 (0.56)	5.43 (0.90)	0.11
Circumference	18.92 (1.59)	23.03 (3.07)	0.02
Area	24.76 (4.81)	35.33 (10.40)	0.07
	Multifocal ($n = 4$)	Miscellaneous ($n = 4$)	*P* value
Length	7.00 (0.61)	8.15 (0.60)	0.04
Width	4.62 (0.56)	5.36 (0.46)	0.09
Circumference	18.92 (1.59)	22.28 (1.38)	0.02
Area	24.76 (4.81)	33.55 (5.29)	0.05

Numeric values are mean (SD) in μm or μm^2, respectively, form factors are dimensionless.

multicentric, and the nodular BCCs including solid and adenoid cystic, the sclerosing BCCs including morpheic, and the miscellaneous forms. These differences were significant when tested in the two-sided *t* test for independent samples (Table 4). The differences in the measurements as well as the calculated form factors comparing the other three groups (nodular BCCs including solid and adenoid cystic, the sclerosing BCCs including morpheic, and the miscellaneous forms) were not significant.

By means of the presented data a quantifiable, significant difference allows a morphometric differentiation of superficial BCCs, compared to all other histological forms. A morphometric differentiation between non-recurring and recurring BCCs was not verified.

DISCUSSION

BCCs generally grow slowly, behave in a relatively benign non-aggressive fashion, and in most of the cases excision is curative.[1,2] A few BCCs grow aggressively and infiltrate deeper structures. These tumours are difficult to delineate and tend to recur after treatment. Metastatic BCCs (which were not considered in this study) are extremely rare and are usually associated with extensive local invasion or multiple local recurrences.[3–6]

Some authors tend to blame recurrences on incomplete removal of BCCs,[12,13] but even after complete removal tumours can recur. Thus far, BCCs have been studied mainly by histopathological techniques. The growth pattern and leading edges, size and shape of cell groups, the presence of palisading and possibly also of a hyaline nature of the stroma, and cellular differentiation including squamous metaplasia seem to be relevant indications of aggressiveness, but do not result in a final assessment.

The clinical variables, such as age and sex of the patient, size of the tumour and its duration, are not significantly correlated with the development of recurrence.[14,15] Histological observations, such as infiltration[16] and ulceration,[17] do not allow accurate prediction of malignancy of an individual BCC. A supplementary method to assess differentiation is the morphometric measurement of cell structures. In these measurements the nucleus is of great importance. The change in its form is often an indication of a malignant potential. The relevance of morphometric analysis of nuclei is well documented in grading carcinoma of the urinary bladder,[18] the prostate,[19] hepatocellular carcinoma,[20] gastric dysplasia[21] and colorectal polyps.[22,23]

De Rosa *et al*.[24] were the first to study nuclear morphometric features of two groups of BCC and compare them with classic cytologic features of the tumour. A group of ordinary BCC was compared morphometrically with a group of aggressive (partly recurrent, partly metastatic) BCC. The variables length, width, circumference and nuclear area and the form factors axis ratio and shape factor were assessed. The mean values differed significantly in length, width, circumference and nuclear area. Length and width were the most relevant distinctive features. As in our study, differences between the mean values of the form factors axis ratio and shape factor were not significant.

In our study, we found no significant morphological differences between recurring and non-recurring tumours, which is in accordance with the absence of histological differences between these tumour types. Even BCCs, described as 'horrifying basal cell carcinoma' by Jackson and Adams,[25] did not differ histologically from harmless BCCs. It is obvious that some BCCs have more aggressive potential than others or, because of their growth pattern, are more difficult to excise. However, these differences cannot be defined either by histological or by morphometric methods.

In our study, the length, width, circumference and area were significantly smaller in the multifocal superficial

BCCs than in the other groups. This reflects the fact that multifocal superficial BCCs show an increased tendency to recur. This is in agreement with Dixon *et al.*[14] and Jacobs *et al.*[26] who described a significantly higher recurrence rate for superficial multicentric BCCs and infiltrating and morpheiform BCCs than for nodular BCCs. With an excision margin of >0.75 mm, 22% of superficial BCCs recurred, compared with 4% of nodular BCCs. The cause is not a different biological behaviour of the tumours, but a more difficult and therefore often inadequate excision.

It can therefore be stated that form factors in general do not differentiate between primary and recurrent tumours nor certain histological types of BCCs and are of no benefit for the assessment of BCCs. This emphasises the estimation of the special oncologic position of the BCCs regarding their low malignancy and makes it necessary to search for additional methods for future investigations in order to define the aggressiveness of BCCs.

CONCLUSIONS

We found differences in specific nuclear morphometric features which discriminated superficial BCCs from all other forms. A differentiation between non-recurring and recurring tumours was not proved. Morphometric studies may be useful in the differentiation of these tumours, but are only an initial step in the study of BCCs. Morphometric examination of larger series, coupled with DNA analysis, may improve our knowledge and allow the prognostic evaluation of this group of neoplasms.

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