DIFFERENTIATION BETWEEN MORPHOLOGICALLY AGGRESSIVE AND NON-AGGRESSIVE BASAL CELL CARCINOMA BY AgNOR STAINING

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ABSTRACT

Argyrophilic staining of nucleolar organizer regions (AgNOR) has been considered to be useful in the diagnosis and prognostic evaluation of different cutaneous tumors. In order to evaluate the role of the AgNOR technique in discriminating aggressive from non-aggressive basal cell carcinoma (BCC), paraffin-embedded histologic sections from 30 cases of aggressive BCC (BCC2) and 30 cases of non-aggressive BCC (BCC1) were investigated for nucleolar organizer regions (NOR), and these cases were categorized on the basis of histologic criteria. The means of AgNOR counts were higher in BCC2 than BCC1 without overlapping in the range of NOR numbers. In BCC2 the majority of the dots had irregular borders and were of varying sizes, whereas in BCC1, the dots had regular borders with little variation in size.

The means of AgNOR counts were 4.81 with an SD (standard deviation) of 0.88 for aggressive and 2.39 with an SD of 0.61 for the non-aggressive group. The difference was statistically significant (p value= 0.000).

The AgNOR number, their configuration and size, may provide information to be useful for recognition of aggressive BCC. Considering histologic criteria, AgNOR counting and clinical features, we can predict the behavior of most BCC tumors.

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INTRODUCTION

Basal cell carcinoma (BCC) is one of the most common cutaneous tumors accounting for approximately 70% of all malignant neoplasms of the skin. This tumor is seen almost exclusively on hair-bearing skin especially on the face, generally occurs in adults and is more common in males than females.[1]

BCC is truly invasive in only a small proportion of cases, in which it penetrates the deep structures, destroying them and sometimes giving rise to metastatic spread.

Because of the difference in management and prognosis of aggressive and non-aggressive BCC, it is necessary to differentiate these two groups.[2]

Some authors have postulated that by utilizing histologic criteria, pathologists can confidently diagnose the majority of aggressive cases and so adequate treatment and careful followup become feasible,[3] but until now traditional histologic diagnostic criteria have failed to unequivocally discriminate between BCC1 and BCC2. Consequently over the past two decades, many morphological and biological factors have been investigated including NOR status, overexpression of P53 protein, bcl_2 protein expression[4] and tumor angiogenesis.[5]

Silver staining of nucleolar organizer regions

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(AgNOR) is a technique that can help to distinguish BCC1 from BCC2. This technique has been employed for tumors of various organs including the skin. This method has been used for separating benign cutaneous lesions from malignant ones, for example malignant melanoma from benign nevocellular nevi.[6]

A quantitative assay of AgNOR and perhaps their distribution pattern can provide information useful to recognize BCC2 and then may be helpful in their prognostic prediction.[7]

MATERIAL AND METHODS

Selection of cases

We reviewed Hematoxylin and Eosin sections from 400 cases of cutaneous BCC surgically treated in the Ali Asghar and Shahid Faghihi Hospitals of Shiraz Medical University from 1992 to 1999. Among these, 30 cases of BCC1 and 30 cases of BCC2 were chosen on the basis of some histologic criteria.

The histologic criteria used for diagnosis of BCC2 were: loss of peripheral palisading, loss of artifactual cleft, increased number of mitosis and abnormal mitosis, pleomorphism, spiky stromal invasion and hyalinization of the stroma. For cases to be considered as BCC2 more then two of the above criteria had to be present in 75% of the tumoral tissue. The morphologic criteria used to identify BCC1 were well formed nests with typical peripheral palisade arrangement of basaloid cells surrounded by fibrous stroma. Routine section of paraffin blocks were taken and stained by AgNOR method, then the mean number of AgNOR dots were calculated.

AgNOR staining procedure

Solutions for AgNOR staining are:

Solution A: 50g silver nitrate dissolved in 100 mL distilled water.

Solution B: 2g gelatin and 1 mL formic acid in 100 mL distilled water.

Solution C: 2 parts of solution A and 1 part of solution B.

Staining procedure:

1- Take 5μ thick sections of paraffin embedded tissue.

2- Dewax sections in xylene.

3- Hydrate sections through ethanol to water solutions.

4- Incubate the sections in freshly prepared solution C for 60 minutes in a sun unexposed dish at room temperature.

5- Wash in distilled water for 1 minute.

6- Dehydrate and mount the sections.

AgNOR dot enumeration

After this treatment, NORs are visualized as distinct black intranuclear dots (Figs. 1 and 2). Counting was done at first by one observer and then by two observers with a double-head microscope for an immediate interobservational control. In each slide, at least 50 nuclei of tumoral cells were examined using a 1000× oil immersion lens and the mean number of AgNOR dots was calculated in each slide. In some of the slides heavy cytoplasmic melanin which was stained as black dots caused difficulty in count-
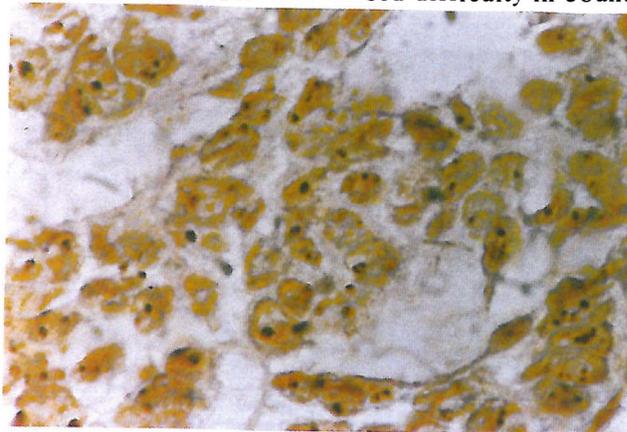

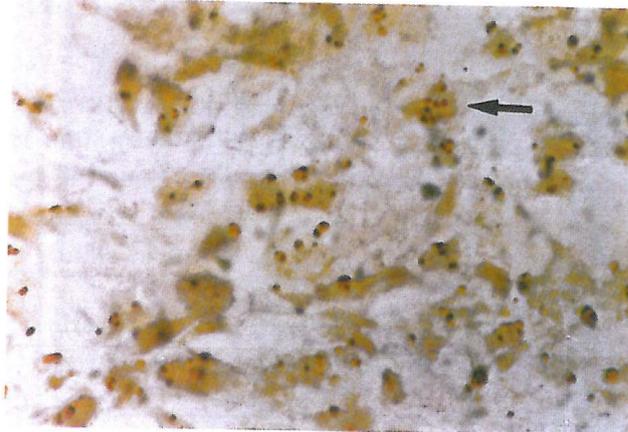**Fig. 1.** BCC 1, AgNOR staining (×1000). The tumor cells contain 1-2 AgNOR dots.

Fig. 2. BCC 2, AgNOR staining (×1000). Some of the tumor cells contain 6-7 AgNOR dots (arrow).

ing AgNOR dots, so areas devoid of melanin pigmentation were chosen for counting.

RESULTS

The number of NOR dots counted in BCC1 ranged from 1.49 to 3.73 with a mean and SD of 2.39 and 0.61 respectively. In BCC2, counts ranged between 3.82 and 7.32 with a mean and SD of 4.84 and 0.88 respectively.

No overlapping was found between the mean and ranges of NOR number in all cases of both groups. The

Table I. Clinical findings in BCC1 and BCC2 cases.

		BCC1	BCC2
Sex	M	19	22
	F	11	8
Age (years)		9-75	14-80
Site	CF	15	17
	O	15	13
Shape of lesion		N= 9 U= 8 P= 7 F= 6	N= 6 U= 16 P= 5 F= 3

Abbreviations;
CF: Central area of face   O: Other
N: Nodule   U: Ulcer
P: Papule   F: Flat

difference between the two groups was statistically significant (p value= 0.000). NOR dots were counted in normal epidermal cells of some of the slides, and the dots ranged from 1-3 per basal cell nucleus.

Clinical findings of the patients are presented in Table I. The mean ages were 50.39 years and 61.5 years for BCC1 and BCC2 groups respectively. Ulcerative lesions were found in more then half of the patients in the BCC2 group but in less than one-third of the patients in the BCC1 group.

No statistically significant differences were found between the two groups with respect of site, sex distribution and type of the lesions.

Unfortunately, only one-third of the patients in each group had follow-up records which were up to 3 years and only one case in the BCC2 group has had a recurrence.

DISCUSSION

This study showed a significantly higher mean AgNOR count in BCC2 than BCC1 tumors categorized on the basis of histologic criteria.

De Rosa et al. (1992) applied the AgNOR method to 11 cases of aggressive BCC (BCC2) and 11 cases of non-aggressive BCC (BCC1) of the skin. They classified BCC to non-aggressive (BCC1) and aggressive (BCC2) types on the basis of H&E histologic criteria.

The histologic criteria for diagnosis of BCC2 were loss of peripheral palisading, loss of artifactual cleft, increased number of mitosis and abnormal mitosis, marked pleomorphism and hyperchromasia, spiky stromal invasion and hyalinization of stroma.

Their results show a mean AgNOR count of 6.56 with an SD of 1.98 for non-aggressive BBC (BCC1) and 9.84 with an SD of 2.12 for aggressive BCC (BCC2). The authors concluded that a quantitative assay of AgNOR and perhaps their distribution pattern may provide information useful to recognize BCC2.[7]

Our study showed similar results, although the counts were lower than those reported by these authors. Considering the fact that the same fixation and the same techniques have been used, this difference could be due to the difference in the method of enumeration of dots in these two studies. The dots forming clusters have been considered as one dot in our study. For differentiation between BCC1 and BCC2 cases, we used the same H&E criteria mentioned above.

This method also has been used for separating benign from malignant cutaneous lesions, for example malignant melanoma from benign nevocellular nevi.[8]

Howat et al. (1990) applied this method to 30 benign nevocellular nevi (BN), 30 dysplastic nevi (DN) and 30 malignant melanomas (MM) of the skin. Their results showed a significant difference between counts of MM and those of BN and DN, despite some overlap. They concluded that the AgNOR technique may be of value in helping to differentiate MM from DN, but is unlikely to be of help in separating DN from BN.[3]

Kanitakis et al. (1992) applied the AgNOR method to 20 cases of squamous cell carcinoma (SCC) of the skin and 16 cases of keratoacanthoma (KA). Their results showed significantly higher AgNOR counts in SCC (6.929+/-0.91) compared to KA (3.80+/-1.62).[9]

Rosa et al. (1997) used this technique for differentiation between ameloblastoma and BCC of the skin. The mean AgNOR counts were lower in ameloblastoma (1.652+/-0.032) compared to those in BCC (2.354+/-0.054). They concluded that although ameloblastoma and BCC are neoplasms with similar growth patterns, they have cell populations with significantly different AgNOR patterns.[10]

Another method which has recently been used to differentiate BCC1 from BCC2 is tumor angiogenesis assessment.[5]

Correlation between AgNOR counts and other cell proliferation indices (e.g., S-phase fraction by DNA flowcytometry and Ki-67 immunohistochemistry) have previously been reported.[4]

The present study showed no statistically significant differences between BCC1 and BCC2 groups with respect to sex, site of lesion and age distribution as well as appearances of the lesions. Due to insufficient followup records, we could not correlate our results of AgNOR counts with more important clinical criteria of aggressiveness such as occurrence of relapse or metastasis.

In conclusion, AgNOR count has significant correla-

tion with histologic criteria used for differentiation between BCC1 and BCC2. Most of the selected cases of BCC2 correlate with the ulcerative type of the lesion which is usually associated with aggressive behavior.

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