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Setting of reliable immunohistochemical criteria for the recurrence of nodular basal cell carcinoma

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Introduction. Basal cell carcinoma (BCC) is one of the most prevalent skin neoplasms with increasing incidence. The grade of BCC malignancy is highly variable and depends on the invasiveness and recurrence potential. The study was aimed at identification of immunohistochemical (IHC) determinants of BCC recurrence.

Materials and methods. The comparative study encompassed 10 cases of primary BCC and 10 cases of recurrent BCC. The panel of immunohistochemical targets included p53, CK8/18, Bcl-2, CK19, Collagen type IV, Desmin, CD8, Ki-67, Vimentin, VEGFR, EGFR and AR.

Results. Diffuse expression of vimentin (characteristic of both primary and recurrent BCCs and clearly indicating the border between the tumor stroma and the surrounding dermis) in the recurrent tumors was twice as strong as in the primary tumors. Immunohistochemistry for collagen type IV revealed different nature of the basement membrane alterations in the primary and recurrent tumors. A two-fold increase in the intensity of angiogenesis observed in the recurrent tumors was accompanied by a more than two-fold significant increase in the androgen receptor protein expression.

Conclusion. Increasing grade of BCC malignancy is associated with the immunohistochemically revealed reinforcement of the stromal and vascular components of the tumor, as well as progressive destruction of the basement membrane along with the increased expression of androgen receptor protein by tumor cells.

Keywords: basal cell carcinoma, recurrence, immunohistochemistry

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Иммуногистохимические критерии рецидивирования базально-клеточной карциномы нодулярного строения

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Введение. Базально-клеточная карцинома является одним из самых частых новообразований кожи и имеет тенденцию к повышению уровня заболеваемости. Среди критериев злокачественности этой группы новообразований нужно отметить ее инвазивный потенциал и способность к рецидивному росту. Цель исследования – определить иммуногистохимические особенности стромального компонента базально-клеточных карцином для прогноза рецидивирования.

Материалы и методы. В исследуемую группу вошли 10 случаев первичных и 10 случаев рецидивных базально-клеточных карцином. Для иммуногистохимического исследования использовали панель антител, включающую в себя следующие маркеры: p53, CK8/18, Bcl-2, CK19, Collagen IV, Desmin, CD 8, Ki-67, Vimentin, VEGFR, EGFR, Androgen receptor.

Результаты. По данным гистологического и иммуногистохимического исследования экспрессия виментина, носящая диффузный характер в первичных и рецидивных опухолях, не только позволила

четко определить границу опухолевой стромы и окружающей дермы, но и в 2 раза отличалась по количественным значениям. Экспрессия коллагена IV типа показала различный характер изменений базальной мембраны в первичных и рецидивных опухолях. В рецидивных опухолях было установлено усиление ангиогенеза в 2 раза. Экспрессия андрогенов также показала достоверную разницу более чем в 2 раза.

Заключение. По мере нарастания агрессивных свойств базально-клеточного рака кожи возрастает экспрессия антигенов основных волокнистых стромальных структур, увеличивается индекс пролиферативной активности, происходит усиление ангиогенеза.

Ключевые слова: базально-клеточная карцинома, рецидив, иммуногистохимия

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Introduction

Basal cell carcinoma (BCC; synonyms: basalioma, basal cell epithelioma) is the most prevalent malignant tumor of the skin. According to the WHO Classification of Skin Tumors (2018), BCCs (which consist of basaloid cells) are classified as truly malignant as they show uncontrolled growth with infiltration and destruction of the underlying tissues, which also allows classifying them as tumors with locally destructive growth [1]. BCC can develop at any age, but is predominantly seen in patients over 50 [2, 3]. Despite the wide variety of clinical and morphological variants, the prevalent form of BCC (42.8%) is nodular [4]. The nodular variants constitute 60–80% of all basal cell skin cancers [1].

The recurrence of tumors of this subgroup varies from 2–7% to 13.6–21.6% and is practically independent of the method of treatment during the first year after the therapy. The tumor recurs in 40% of the patients within 10 years after the treatment, and the cases of long-term (measured in years) ineffective therapy for basalioma are common. The multiplicity of factors for basalioma recurrence have been described, including large tumor size and the infiltrative-ulcerative type of growth with deep invasion and perineural expansion. Localization of the tumor is considered no less significant, with skin of the nose, nasolabial triangle, or lower eyelid with the involvement of the intermarginal strip being most susceptible to basalioma recurrence. The recurrence rate also increases with the development of a malignant focus against the background of precancerous skin lesions. Certain functional correlates of BCC recurrence propensity have been described as well, e.g. the elevated expression of vascular endothelial growth factor and its cognate receptor (VEGF/VEGFR) by tumor cells accompanied by high proliferative activity (indicated by increased expression of and Ki-67) and altered expression of major regulators of apoptosis (p53, bcl-2). Nevertheless, none of the suggested mechanisms provides unambiguous explanation for the emergence of a localized process of the persistently recurrent nature [5–10]. Such a gap in knowledge makes the diagnosis and treatment of BCC a high-priority interdisciplinary focus of clinical dermatology, oncology and pathomorphology.

In this comparative study, we aimed at identification of immunohistochemical (IHC) determinants of BCC recurrence.

Materials and Methods

The study encompassed 10 cases of primary BCC and 10 cases of recurrent BCC in patients of the Center for Diagnostics and Treatment of Skin Tumors of the Privolzhsky Research Medical University. Surgical treatment with the excision of a primary or recurrent tumor was based on clinical indications. The total sample included 8 men and 12 women, aged 72.5±13.1 years on average. In the group of patients with primary tumors, no recurrence episodes were observed for 3 years after the surgery. In the group of patients with recurrent BCCs, the recurrence emerged within 1–2.5 years after the surgery.

The surgical specimens were fixed in 10% formalin. The resection was performed with mandatory inking of the margins to determine the presence or absence of invasive growth. Routine histological processing of the material was implemented with the use of Excelsior ES tissue processor (Thermo Scientific, USA). The dehydrated specimens were embedded in paraffin with the use of HistoStar embedding workstation (Thermo Scientific), sectioned with the use of HM 325 rotary microtome (Thermo Scientific) at a 4–6 μm thickness and stained with H&E with the use of Gemini AS automated slide stainer (Thermo Scientific).

The IHC staining was carried out in a BOND-MAX automated module (Leica Biosystems, Germany).

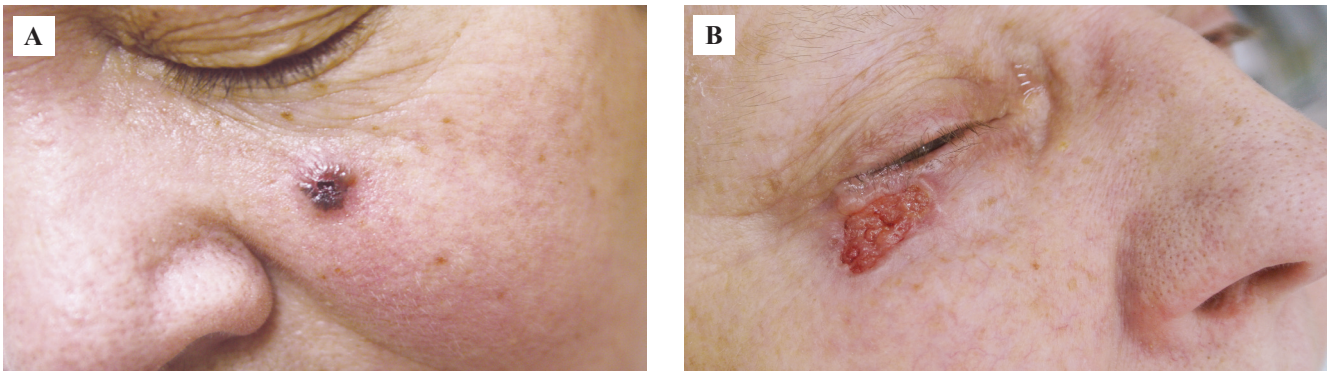

Figure 1. Clinical images of (A) – nodular basal cell carcinoma, (B) nodular basal cell carcinoma with ulcus rodens
Рис. 1. Клиническое изображение. А – узловая форма базально-клеточного рака, В – ulcus rodens при узловой форме базально-клеточного рака

The panel of antibodies used for IHC is given in Table 1.

The morphometry was carried out with the use of Leica DM2500 optical microscope (Leica, Germany).

The expression of antigens was evaluated by counting positively stained cells in 10 fields of view at a ×400 magnification. The expression indexes were calculated as the number of positive cells × 100/total cell number.

The Mann-Whitney U-test was used to identify differences in the studied parameters between independent groups. The results are represented as median values (Me, Q50%) complemented with Q25% and Q75% values.

Table 1 | Таблица 1

The panel of antibodies used in the study | Панель антител, используемых в исследовании

	Title | Название	Clone | Клон	Manufacturer | Производитель
Mouse | Мышиные	p53 CK8/18 Bcl-2 CK19 Collagen IV Desmin CD 8	Do7 5D3 124 Ks19.1 CIV22 D33 4B11	Novocastra, UK Novocastra, UK Dako, Denmark Lab Vision Corporation, USA Cell Marque, USA Lab Vision Corporation, USA Novocastra, UK
Rabbit | Кроличьи	Ki-67 Vimentin VEGFR EGFR Androgen receptor	SP6 SP20 Poly EP38Y poly	Diagnostic Biosystems, Netherlands Lab Vision Corporation, USA Diagnostic Biosystems, Netherlands Lab Vision Corporation, USA Lab Vision Corporation, USA

Results and Discussion

In the studied cases, the nodular form of basal cell carcinoma was most frequently localized in the skin of the face and scalp, less often in the skin of the trunk, in 20% and 80% respectively. The tumor was more frequently represented by the single nodular pink or gray-pink formation of a hemispherical shape, the peripheral edge of which was surrounded by a ridge consisting of small "pearls" (Figure 1A) [11, 12]. Erosions or ulcers covered with a dark brown hemorrhagic crust were repeatedly found in the center of the tumor. In case of extensive ulceration

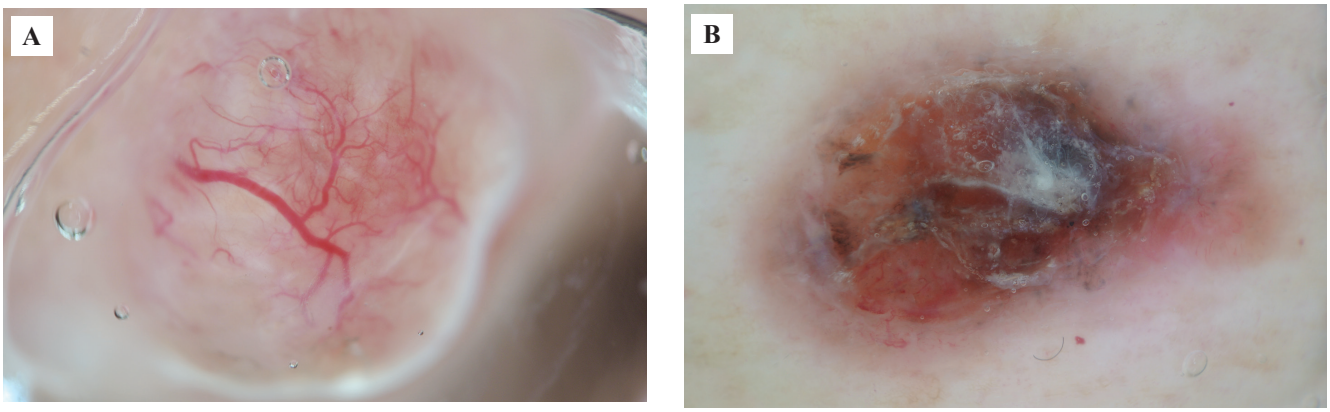

Figure 2. Dermatoscopy images of (A) – nodular basal cell carcinoma, (B) – pigmented nodular basal cell carcinoma
Рис. 2. Дерматоскопическое изображение. А – узловая форма базально-клеточного рака, В – пигментированная узловая форма базально-клеточного рака

with central necrosis, it was diagnosed as an ulcerative form or a corroding ulcer (ulcus rodens) (Figure 1B).

The dermatoscopy images of nodular BCCs in the studied cohort revealed the presence of large tree-like vessels of a bright-red color, the areas of erosion or ulceration covered with a dark-brown hemorrhagic scab, and bluish-gray ovoid structures and globules with pigmentation (Figure 2 A, B). Upon macroscopic examination of the surgical specimens, primary BCCs were distinguished by large size of nodular formations and smooth surface exhibiting telangiectasias. In primary BCCs, the entire tumor focus was represented by a solitary nodule with tuberous surface or an ulcerated core walled with a scalloped array of nodulary elements (Figure 3).

Histological examination of the tumors revealed small basaloid cells – intensely stained, with compact rounded or oval nuclei. The H&E staining revealed neither specific structural features of the chromatin nor nucleoli; the cytoplasm, usually basophilic, was scarce. The periphery of the foci was occupied by zones of larger prismatic cells with oval, slightly elongated nuclei and characteristic palisade arrangement, regarded as a key histological determinant of BCC. The tumor cells showed no intercellular bridges but contained mitotic figures. The fibro-cellular connective tissue stroma of the tumor was organized in bundles which divided the cellular complexes into lobules separated from the metachromatic mucoid substance rich in glycosaminoglycans and comprising the infiltrates of lymphocytes, basophils and plasma cells. In slides, the epithelial tumor complexes were frequently separated from the stroma by characteristic clefts classified as retraction artifacts typical for BCC (Figure 4).

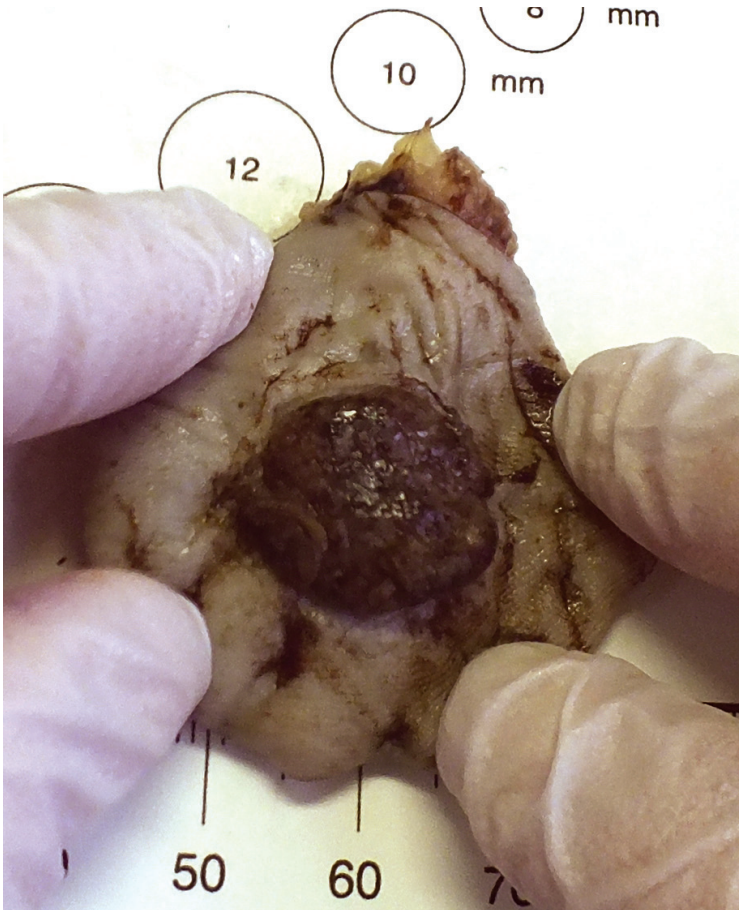

Figure 3. Gross appearance of nodular basal cell carcinoma
Рис. 3. Макроскопическая картина базально-клеточной карциномы узлового строения

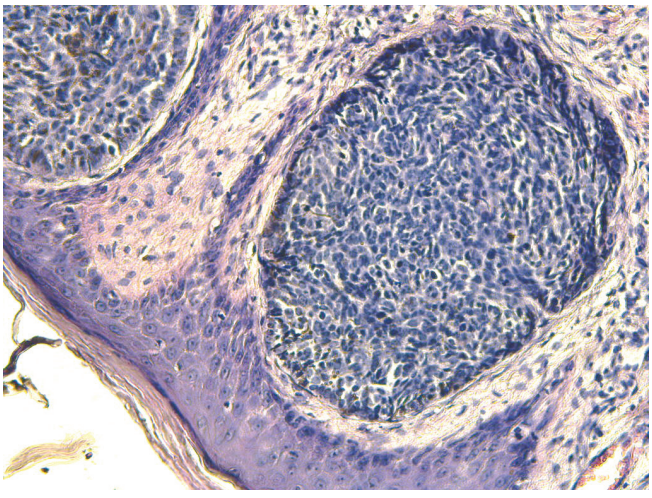

Figure 4. Routine histology of nodular basal cell carcinoma. H&E, magnification ×200
Рис. 4. Гистологическая картина узловой формы базально-клеточной карциномы. Окраска гематоксилином и эозином, ×200

The overall dimensions of the neoplasms ranged from 0.5 to 2.5 cm. The invasion was confined to the dermis in all cases. The distance from the tumor to the closest resection margin ranged from 0.1 to 0.15 mm. The resection margins were tumor-negative in all cases, and no signs of perineural invasion were observed. The groups of primary and recurrent tumors were further compared by IHC criteria (Table 2).

Proliferative activity of primary and recurrent BCCs was characterized by uniform distribution of Ki-67-positive tumor cells which amounted to 26% in primary tumors and 46% in recurrent tumors ($p = 0.0023$, Figure 5). The vimentin expression was diffuse and provided a clear demarcation of the border between the tumor stroma and the surrounding dermis. The content of vimentin-positive cells in primary BCCs (20%) was lower than in recurrent BCCs (47%, $p = 0.026$, Figure 6). We do not as-

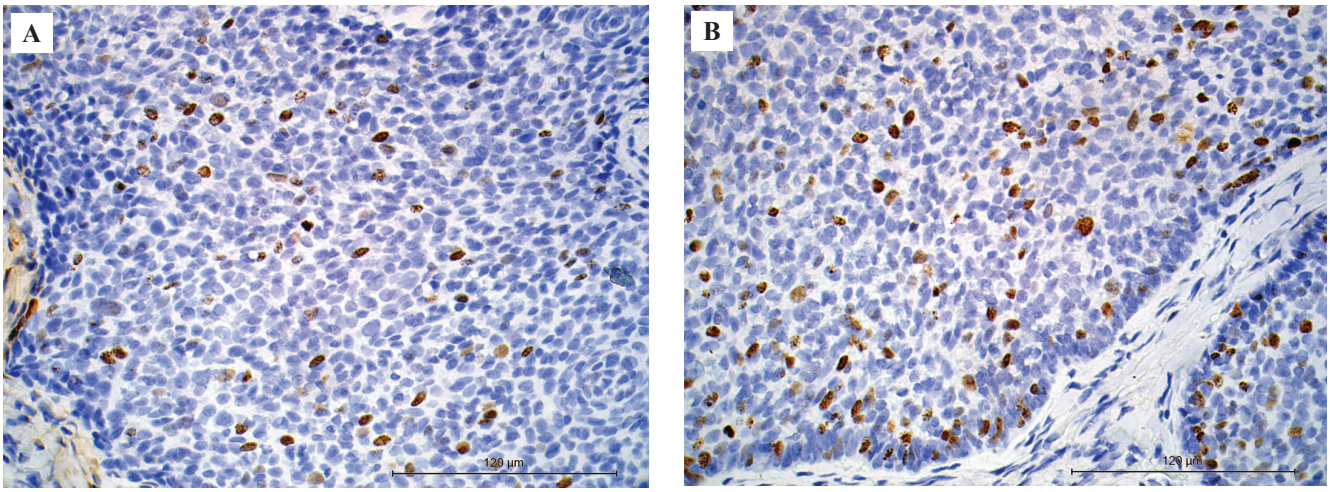

Figure 5. Ki-67 protein expression in (A) – primary BCCs, (B) – recurrent BCCs. Immunohistochemistry, magnification ×400
Рис. 5. Экспрессия Ki-67 в первичных (А) и рецидивных (В) опухолях. ИГХ окрашивание, ×400

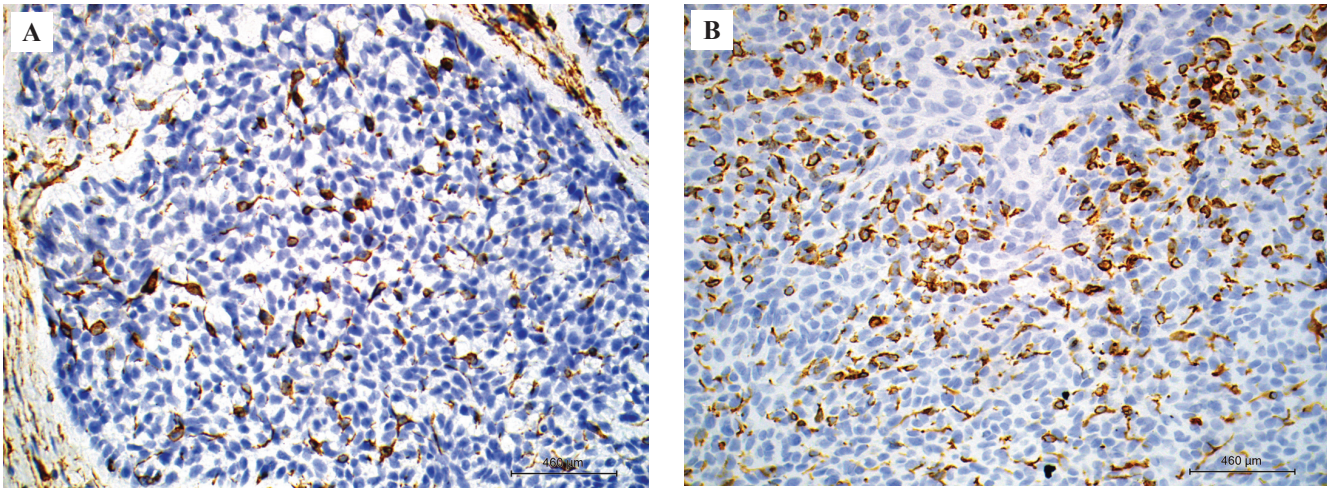

Figure 6. Vimentin protein expression in (A) – primary tumors, (B) – recurrent tumors. Immunohistochemistry, magnification ×400
Рис. 6. Экспрессия виментина в первичных опухолях (А) по сравнению с рецидивными опухолями (В). ИГХ окрашивание, ×400

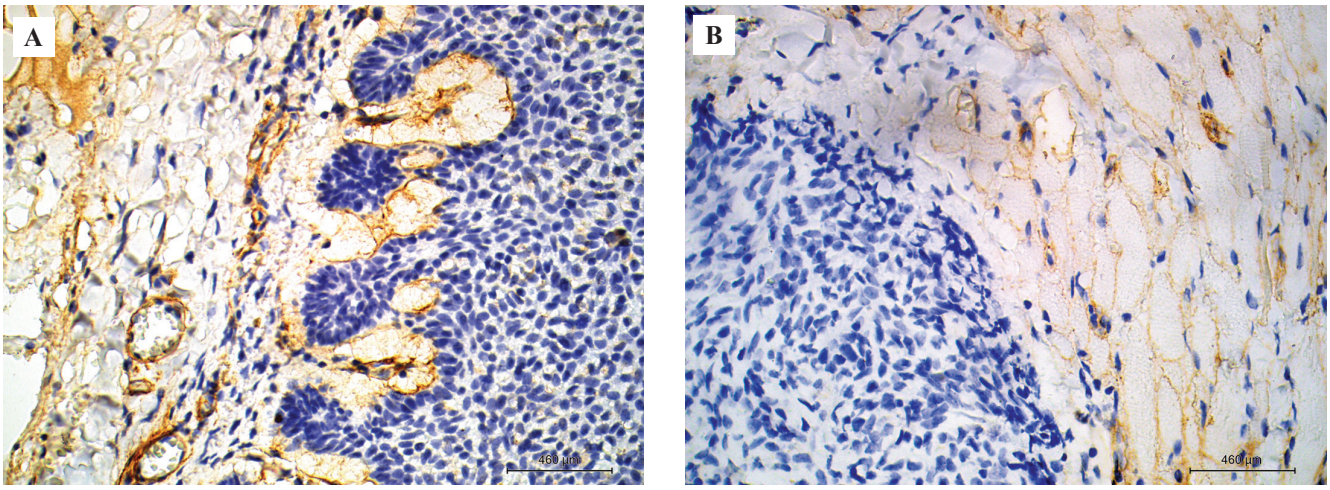

Figure 7. IHC image for collagen type IV revealing (A) – ruptures of the basement membrane in primary tumors, (B) – destruction of the basement membrane in recurrent tumors. Immunohistochemistry, magnification ×400
Рис. 7. Коллаген IV типа. Разрыв базальной мембраны в первичных опухолях (А), отсутствие базальной мембраны в рецидивных опухолях (В). ИГХ окрашивание, ×400

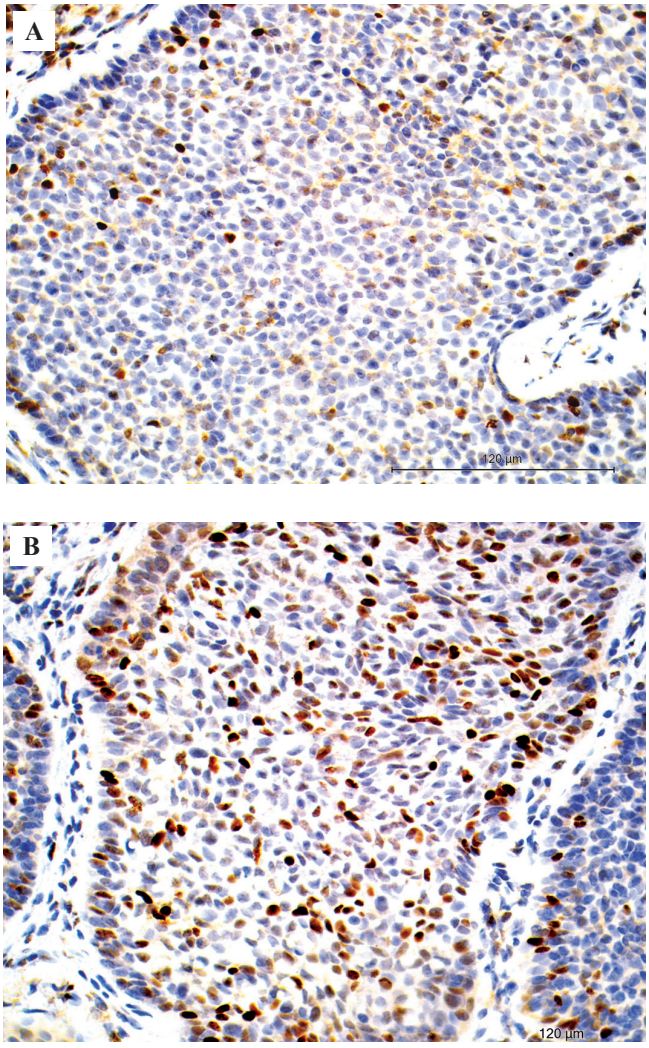

Figure 8. AR protein expression in the nuclei of tumor cells (A) – in primary tumors, (B) – in recurrent tumors. Immunohistochemistry, magnification ×400.

Рис. 8. Экспрессия рецепторов андрогенов в ядрах опухолевых клеток. А – в первичных опухолях, В – в рецидивных опухолях. ИГХ окрашивание, ×400

Table 2 | Таблица 2

Comparative immunohistochemical characteristic of primary and recurrent basal cell carcinomas (BCC) | Сравнительная иммуногистохимическая характеристика первичного и рецидивного базального-клеточного рака (БКР)

IHC marker | ИГХ маркер	Primary BCC (n=10) | Первичный БКР (n=10)	Recurrent BCC (n=10) | Рецидивный БКР (n=10)
p53	35±4,2%	35±3,4%
Ki-67	26±2,9%	46±3,7%
Bcl-2	+	+
CK8/18	+++	+++
CK19	–	–
Collagen type IV | Коллаген IV типа	+ (basal membrane with ruptures) | (базальная мембрана с разрывами)	++ (there is no basal membrane) | (базальная мембрана отсутствует)
Vimentin	20±6,1%	47±5,3%
Desmin	–	–
EGFR	26±1,9%	46±2,4%
CD 8	–	–
Androgen receptor | Рецепторы андрогенов	15±3,9%	42±4,2%
VEGF	12±4,8%	32±4,2%

sociate the expression of vimentin with the presence of melanocytes, as the study involved unpigmented forms of the tumor.

IHC with antibodies to collagen type IV revealed ruptures of the basement membrane in primary tumors and its complete absence in recurrent tumors (Figure 7).

The content of androgen receptor-positive cells in the nodules of primary BCCs (15%) was lower compared with recurrent BCCs (42%, p = 0.002, (Figure 8).

IHC for vascular endothelial growth factor receptor (VEGFR) revealed diffuse staining of clustered tumor cells in all cases; the increased content of positive cells in recurrent BCCs (32% compared to 12% in the primary tumors) indicates the elevated rates of angiogenesis in the recurrent neoplasms.

Conclusion

The study identified significant IHC determinants of BCC recurrence, including the increased Ki-67 index, progressive destruction of the basement membrane, and the increased expression of vimentin, EGFR, VEGFR and notably the androgen receptor (AR).

No differences in the expression of apoptosis-related regulatory proteins (p53, bcl-2) were observed between the groups. The expression of CK8/18 was ubiquitous; the staining for CK19, Desmin and CD8 was weak and showed no difference between the groups. The study revealed no clear correlation between the size of BCC and its aggressive properties. The possibility of using AR as a diagnostic marker or therapeutic target in BCCs requires further investigation.

Author contributions
N.Yu.O, I.L.S. and O.E.G. conceived the study and designed the experiments;
O.E.G., N.Yu.O. and D.V.D collected the data and performed the analysis;
N.Yu.O., D.V.D., I.L.S. and O.E.G. wrote the manuscript;
D.V.D. edited the manuscript.

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