

ELECTRON MICROSCOPY STUDY OF NODULAR BASAL CELL CARCINOMA

Gurgas Leonard¹, Doru-Popescu Nelu², Hangan Tony¹, Chirila Sergiu¹, Moroianu Olimpia³, Roșoiu Natalia^{1,4}

¹ Faculty of Medicine, University "Ovidius" of Constanta

² Naval Medical Centre Constanta

³ Doctoral School, University "Ovidius" of Constanta

⁴ Academy of Romanian Scientists, Bucharest

Leonard Gurgas

Aleea Meduzei, nr. 10, bl. 22 Est, sc. B, et. 2, ap. 16,

Năvodari, Constanța, Romania

email: leonard.gurgas11@gmail.com

phone: +40 751480155

ABSTRACT

Abstract. The electron microscopic study represent the changes in the skin layers in the malignant tumor; the nodular basal cell epithelioma. Comparisons were made between normal cells found in the normal skin at the periphery of the tumor, the cells located near the tumor and the tumors located in the depth of the carcinoma. The microscopic analysis of the tumor formation revealed the characteristics of the pigmented nodular basal cell epitheliu. To the exterior of the carcinoma were evidenced numerous globular formations limiting the peripheral extension of the tumor, which explains its evolution in years.

Keywords: Electron microscopic, basal cell carcinoma, nodular, tumoral

Introduction

Basal cell epithelioma is a malignant tumoral cell origin in epidermal basal cells (cutaneous, anexal) (1). Basal cell carcinoma does not affect mucous membranes, has local invasiveness and metastasis (2). Metastasis in this type of carcinoma is very rare and only in exceptional cases (3).

Triggers: sun exposure (4), ultraviolet radiation, X radiation, As (5), genetic factors (rare in africans), various scars. UV rays also have a predominant role in the etiology of basal cell carcinomas, a finding that the clinical signs of chronic sun damage to the skin are the most

potent predictors of this type of epithelioma (3,6). Tumor cells are incapable of keratinization, retaining their ability to divide (7). Basal cell carcinoma has a globular surface and has basalomatous pearls with translucent surface, telangiectasis and sometimes central ulceration (8). It is noted, for all types of skin cancer, a high incidence in the population in rural areas (9). It is also noteworthy the prevalence of carcinoma in the elderly population compared to the younger age (10).

The electron microscopic study represent the changes in the skin layers in the malignant tumor, the nodular basal cell epithelioma. Comparisons were made between normal cells

found in the normal skin at the periphery of the tumor, the cells located near the tumor and the tumors located in the depth of the carcinoma.

Clinical forms:

- Nodular basal cell carcinoma (it occurs with an important frequency in postmenopausal women) (11,12)
- Planar-scar tissue basal cell carcinoma (scarlat plate delineated by a pearl sponge)
- Superficial basal cell carcinoma (erythmato-squamous plaque margin with small pearls) (13).
- Ulcerated basal cell epithelioma („ulcus rodens” - the rapid and ulcerated node) (14)
- Terebrant basal cell carcinoma („ulcus terebrans” - rapid mutilation ulcer). It should be remembered, for this type of carcinoma that has a great invasiveness in the bone structure, that the recommended treatment is surgery (15).
- Pigmented basal cell carcinoma (brown for rich melanocytic content) (13).
- Morpheiform basal cell carcinoma (sclerotic plaque without pearl edge, does not ulcerate) (16)

The histopathological examination describes in the dermis the keratinocyte epitheliomatous masses, delimited by palisade cells (17).

The present study analyzes the electron microscopic structure of a cutaneous tumor formation, which highlights the characteristic features of nodular pigmented basal cell epithelium.

Material and method

The electron microscopic study was performed on a no. of 35 sections of 50-100 nm of a clinically diagnosed tumor cell „Pigmented, nodular basal cell carcinoma” and a number of 28 normal skin sections located at the edge of the carcinoma. The magnitude of the images varied between 4800 x and 49,000 x.

Sample preparation:

To prepare the samples, the modified Jastrow method was used:

- Immersion of samples in 2.5% glutaraldehyde solution buffered with 2% paraffolmaldehyde in 0.1 M sodium phosphate buffer

(Sorensenbuffer) pH 7.2 - 7.4.

- Storage of samples overnight at + 4 ° C.
- Washing 3x15 min. In 0.1 M Sorensen sodium phosphate buffer + 0.1 M sucrose.
- Postfixation 90 minutes in 2% osmium in TF, pH 7.4, + 4 ° C.
- Washing dec3x15 min. In 0.1M sodium phosphate buffer (Sorensen), pH 7.4.
- Dehydration 2x15 min. With 50% acetone (in distilled water).
- Overnight contrast in acetone 70% + 0.5% uranyl acetate + 1% phosphorus acid at + 4 °C.

Dehydration:

- 2 x 15 min. with 80% acetone.
- 2 x 15 min. with 90% acetone.
- 2 x 15 min. with 96% acetone.
- 3 x 20 min. with 100% acetone.
- 2 x 15 min. with propylene oxide.
- 30 min 2: 1 propylene oxide mixture Epon.
- 30 min 1: 1 propylene oxide mixture Epon.
- 30 min 1: 2 propylene oxide mixture Epon.
- Epon impregnation overnight at + 4 ° C.
- Taking the sample and placing it in the fresh Epon.
- Incubation for 48 hours at 60 ° C for polymerization.
- Sections at 50-100 nm.
- Washing the sections.

Over contrasting:

- 10 min. in the soil. 8% uranyl acetate.
- 5 min. in the soil. 0.7% ice citrate + 0.9% sodium citrate.
- Dry the grid 15 min. and examination.
- The electronic microscopy used to obtain photographic images is an electronic transmission microscope in the possession of the Faculty of Medicine in Constanta.
- Key technical features:
- Resolution power 210-10 m.
- Range of increments: 35 – 1 200 000.
- Diffraction: 18 - 4300 mm, with ultra-high vacuum and crimoscopy options.
- As an accessory, the 15kV IMV D-31model UPS was used for protection in case of network voltage drops acquired in 2000.

Results

Anucleus(N),melanocyt(M),melanosomes (MZ) and premelanosome like membraneous structure are present in the cytoplasm (Figure 1).

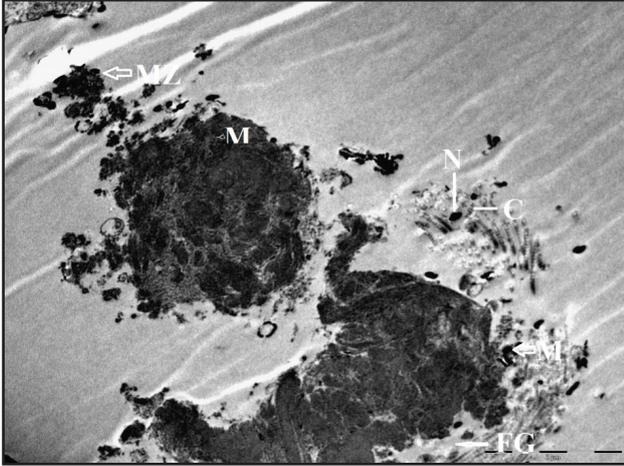


Figure 1 (Magnitude 11000 x)

Intercellular spaces are markedly dilated and globular formations (FG) are noticed in the stroma. Cytolytic changes are present in some parts of the tumor cells (Figure 2).

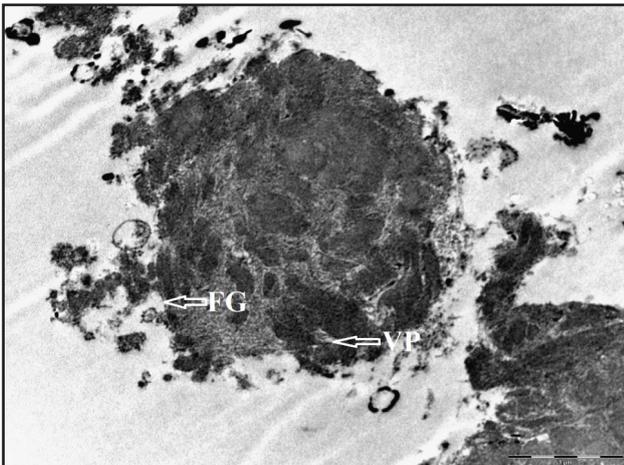


Figure 2 (Magnitude 18500 x)

Pinocytotic vesicles (VP) with or without fine granular amorphous contents and premelanosome like bodies are near the cell membrane of the neoplastic keratinocyte as an individual. The distribution and morphology of both membranous structures suggest an interrelationship. Melanosomes (MZ), melanocyt (M) and glassy amorphous material in the stroma are indicated (Figure 3).

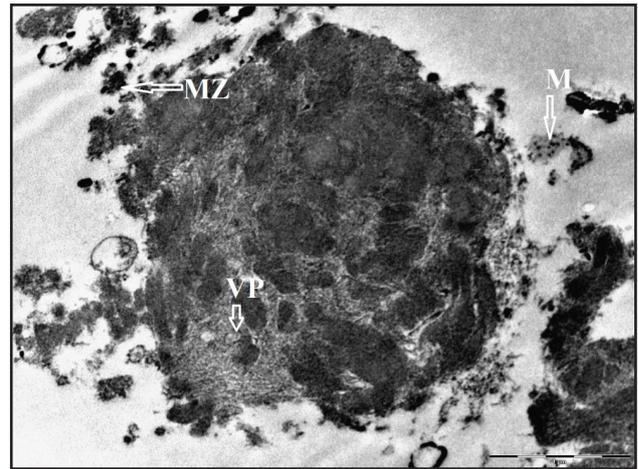


Figure 3 (Magnitude 23000 x)

At 30000x magnitude we can easily distinguish nucleus (N), keratinocyte with keratin fibers (FK) and desmosomes (DZ) that are seen between the melanocyte and the keratinocyte (Figure 4).

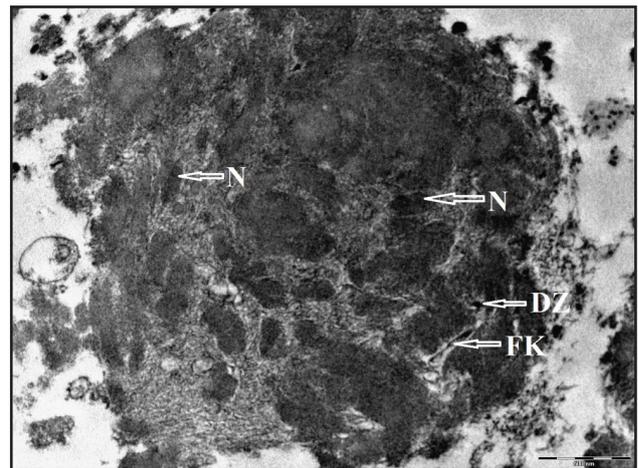


Figure 4 (Magnitude 30000 x)

Melanocyte is rich in tubular rough surfaced endoplasmatic reticula (RE), ribosomes (RZ), mitochondria (MT), globular formations (FG) and desmosomes (DZ). The sections of the tumor peripheral region contain predominantly amorphous globular formations (FG).

The sections of the tumor peripheral region contain predominantly amorphous globular formations (FG) (Figure 5).

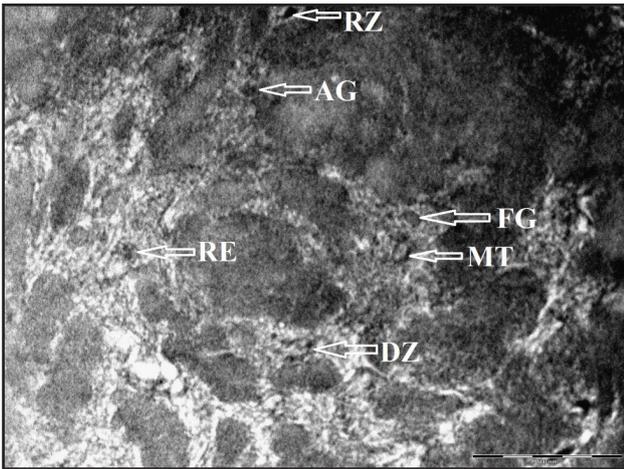


Figure 5 (Magnitude 49000x)

The sections of the free cutaneous area located on the periphery of the tumor reveal normal electron microscopic structure. Pinocytotic vesicles (VP) with or without fine granular amorphous contents and premelanosome like bodies are near the cell membrane of the neoplastic keratinocyte as an individual (Figure 6).

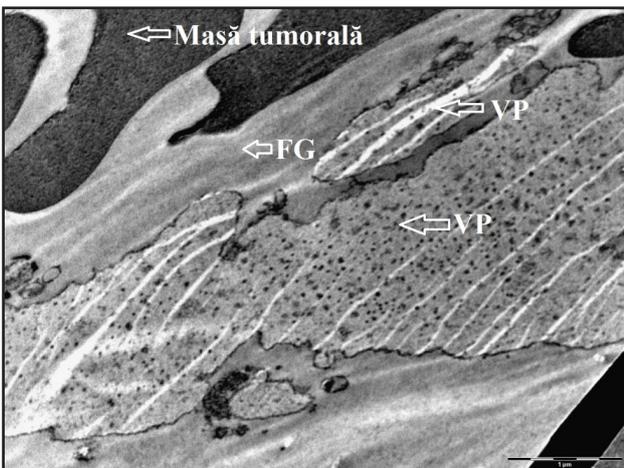


Figure 6 (Magnitude 23000 x)

The sections of the area located on the periphery of the tumor reveal endoplasmatic reticula (RE), mitochondria (MT) and globular formations (FG) with normal electron microscopic structure.

The sections of the free cutaneous area located on the periphery of the tumor reveal keratinocytes with normal electron microscopic structure (Figure 7).

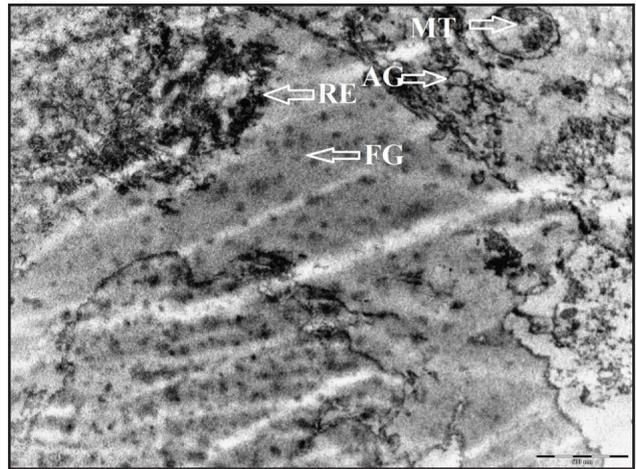


Figure 7 Magnitude (30000 x).

The sections of the free cutaneous area located on the periphery of the tumor reveal keratinocytes (FK), nucleus (N), ribosomes (RZ) with normal electron microscopic structure (Figure 8).

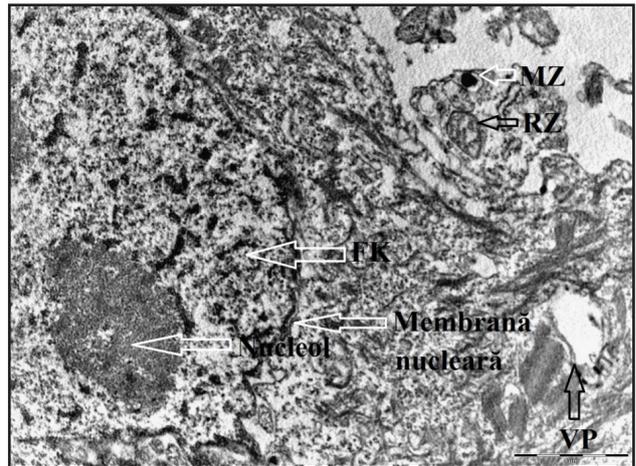


Figure 8 (Magnitude 18500 x)

Discussions

Histologically, the image of nodular basal cell carcinoma shows large nuclei scattered along a well-established node.(18). Present in all types of cancer, stroma also has an important part in the cancerous tissue of nodular basal cell carcinomas. Its role is essential in nutrition, growth and dissemination of the tumor (19). Pigmented basal cell carcinoma is a type of nodular carcinoma, the cause of pigmentation being the phagocytosis of melanosomes by tumor cells and the presence of melanogens in the stroma (20). Initial carcinoma lesions appear

as small pink papules (less than 3mm). The lesion remains on the surface. The telangiectatic vessels are pronounced and become more visible with the lesion extension. (21)

On the sections performed in the deep central area of the tumor (Figure 1, Figure 2, Figure 3, Figure 4, Figure 5) the electron microscopic changes specific to the basal cell epithelium are highlighted:

a) tumor cells located at the periphery of the tumor are ordered in palisade, with large nuclei (N), oval, with a small cytoplasm (C); have rare nuclear monstrosities and cell divisions;

b) melanocytes (M) are large, rich in melanosomes (MZ) and melanogenic pigment, especially disposed at the periphery of the tumor;

c) cells are disposed in tumor masses separated by globular formations (FGs) consisting of stroma composed of amorphous material (22);

d) Visible ultramicroscopic forms are: mitochondria (MT), endoplasmic reticulum (RE), keratin (FK), melanosomes (MZ), desmosomes (DZ), ribosomes (RZ).

Experimentally, by peripheral injection of skin tumors caused to laboratory animals with amorphous substances, they isolate tumor formations from free tissues, and by decreasing blood intake they can be invaded with gradual ischemia.

Conclusions

The microscopic analysis of the tumor formation reveals the characteristics of pigmented nodular basal cell epithelium.

In addition, it notes the systematic existence of numerous globular formations at the periphery of the tumor, consisting of an amorphous substance. These formations limit the peripheral extension of the tumor by junction with normal skin tissue, which explains the slow evolution in years.

References

1. Oanta A. *Curs de Dermatologie pentru studenti*. Brasov: Universitatea "Transilvania" Brasov; 2007.
2. Patrascu V. *Boli dermatologice si infectii*

- sexual-transmisibile. Craiova: Sitech; 2012.
3. Leonard G, Tony H, Sergiu C, Roşoiu N. Environment and gender influence the location of basal cell carcinoma. *Acad Rom Sci Ann Ser Biol Sci Acad Rom Sci Ann -Series Biol Sci*. 2016;5(1):64–72.
4. Lanoue J, Goldenberg G. Basal Cell Carcinoma: A Comprehensive Review of Existing and Emerging Nonsurgical Therapies. *J Clin Aesthet Dermatol* [Internet]. 2016 May [cited 2017 Jan 6];9(5):26–36. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27386043>
5. Puig S, Berrocal A. Management of high-risk and advanced basal cell carcinoma. *Clin Transl Oncol* [Internet]. 2015 Jul [cited 2017 Jan 5];17(7):497–503. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25643667>
6. Corona R, Dogliotti E, D'Errico M, Sera F, Iavarone I, Baliva G, et al. Risk Factors for Basal Cell Carcinoma in a Mediterranean Population. *Arch Dermatol* [Internet]. 2001 Sep 1 [cited 2016 Dec 28];137(9):8–31. Available from: <http://archderm.jamanetwork.com/article.aspx?doi=10.1001/archderm.137.9.1162>
7. Kolarsick PAJ, Kolarsick MA, Goodwin C. Anatomy and Physiology of the Skin. *J Dermatol Nurses Assoc* [Internet]. 2011 Jul [cited 2017 Jan 8];3(4):203–13. Available from: <http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=01412499-201107000-00003>
8. Gheorghe Emma. *Histologie – Lucrări practice – Organe*. Constanta: Ovidius University Press; 2006.
9. Chirilă S, Rugină S, Broască V. Neoplastic Diseases Incidence in Constanta County During. *Ars Medica Tomitana*. 2014;4(79):211–4.
10. Radu Frunză, Sergiu Chirilă, Luiza Dilirici DP. Demografic Aspects for Pigmented Skin Tumors in a Geographical Area with a High Ultraviolet Radiation Level. *Med Connect* •. 2015;10:29–32.
11. Gurgas I L, Hangan TL, Chirilă S, Nicola M, Natalia, Roşoiu. Skin reaction to imiquimod self-treatment in post-menopausal women. A case report. *Rom Soc Ultrason Obstet Gynecol*. 2017;13:[13] 117-119.

12. Ghassan T. Skin - Nonmelanocytic tumors Carcinoma (non-adnexal) Basal cell carcinoma (BCC). *Pathol Inc.* 2016;
13. Condrat I, Tataru A, Haja I. Carcinom bazocelular pagetoid extins apărut după radioterapie - prezentare de caz. *DermatoVenerol (Buc)*. 2015;60: 13-18.
14. Crowson AN. Basal cell carcinoma: biology, morphology and clinical implications. *Mod Pathol*. 2006;19, S127–S147.
15. Gurgas L, Hangan T, Chirilă S, Rosoiu N. Analysis methods of treatment as recurrent factor of basal cell carcinomas. [cited 2017 May 2];51(3). Available from: <http://umbalk.org/wp-content/uploads/2016/12/2016-3-347.pdf>
16. Thaddeus L, Harold P, Carl S. Morpheaform basal cell carcinoma. *Am J Surg*. 1968;116(4):499–505.
17. http://www.spitalul-colentina.ro/scc-ro/scc_files/scc_meniuri/invatamant/scc-i-2_derma2-cursuri.html.
18. Elston DM, Bergfeld WF, Petroff N. Basal cell carcinoma with monster cells. *J Cutan Pathol* [Internet]. 1993 Feb [cited 2017 Jan 6];20(1):70–3. Available from: <http://doi.wiley.com/10.1111/j.1600-0560.1993.tb01253.x>
19. Carcinomul bazocelular – caracteristici generale [Internet]. Available from: <https://newsmed.ro/carcinomul-bazocelular-caracteristici-generale/>
20. Mackiewicz-Wysocka M, Bowszyc-Dmochowska M, Strzelecka-Węklar D, Dańczak-Pazdrowska A, Adamski Z. Basal cell carcinoma – diagnosis. *Contemp Oncol (Pozn)*. 2013;17(4): 337–342.
21. Habif TP. *Clinical Dermatology, a color guide to diagnosis and therapy*. Elsevier; 2010.
22. Gurgas L, Popescu ND, Hangan LT, Chirila S, Rosoiu N. The Evolution of Biochemical Indices After Basal Cell Epithelioma Removal - Case Report. *ARS Medica Tomitana*. 2017 Jan;23(2):99–104.