

CASE REPORT

***In vivo* reflectance confocal microscopy of basal cell carcinoma with cystic degeneration**

CONSTANTIN CĂRUNTU¹⁾, DANIEL BODA²⁾, DANIELA ELENA GUȚU³⁾, ANA CĂRUNTU⁴⁾

¹⁾Department of Physiology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

²⁾Dermatology Research Laboratory, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

³⁾Department of Pathology, "Prof. Dr. Nicolae Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania

⁴⁾"Prof. Dr. Dan Theodorescu" Oral and Maxillofacial Surgery Hospital, Bucharest, Romania

Abstract

Basal cell carcinoma (BCC) is the most common skin cancer, may display various clinical aspects, frequently has subclinical extension, and through local invasion may induce important tissue damage. Reflectance confocal microscopy (RCM) is a modern technique that allows a non-invasive investigation of skin structure with a nearly histological resolution, and may achieve an optimal *in vivo* evaluation of skin tumors in combination with dermoscopy. We report a case of nodular BCC with areas of cystic degeneration in which *in vivo* RCM and dermoscopy evaluation enabled an accurate diagnosis and a detailed preoperative evaluation of tumor margins allowing the optimization of surgical treatment, with best aesthetic and functional results.

Keywords: basal cell carcinoma, cystic degeneration, reflectance confocal microscopy, dermoscopy.

Introduction

Basal cell carcinoma (BCC) is one of the most common cancers in humans and accounts for about 80% of all non-melanoma skin cancers. Men and elderly individuals are more frequent affected, and BCC characteristically develops in sun-exposed body areas such as head, neck and trunk [1–4].

There are various clinical and morphological types of BCC, but the classic form is nodular, which presents as a translucent nodule associated with telangiectasias, located on sun-exposed areas of the skin [2, 5, 6]. A relatively rare subtype of nodular BCC is the cystic form, which often has no clinical particularity and may present as a typical nodular lesion [6, 7]. In histopathological examination, cystic BCC shows various sizes cystic cavities that may result from partial tumoral necrosis or degeneration within tumor masses with typical features of BCC [4, 8–10].

Even if it is a slow growing tumor with a very low metastatic potential, nodular BCC is locally invasive, and may produce extensive destruction of neighboring structures.

The most effective treatment for BCC is surgical excision [11] but, besides the aggressive histological pattern of growth, one major risk factor for extensive local invasion is incomplete excision of the tumor, which occurs more frequently in BCC with subclinical extension or indistinct borders [12]. Thus, careful control of excision margins may increase the cure rate with maximal preservation of uninvolved tissue.

Dermoscopy examination of the lesions increases diagnostic accuracy and allows preoperative evaluation of tumor margins, even if BCC may exhibit a large variety of dermoscopic aspects [13–17].

Reflectance confocal microscopy (RCM) is a modern technique that allows a non-invasive investigation of skin structure with a nearly histological resolution and may be a new approach for the *in vivo* diagnosis and defining the margins of BCC [18–23].

Here we describe the case of a patient with a nodular lesion on the right supraclavicular region for which dermoscopy and RCM examination enabled an accurate *in vivo* diagnosis and a careful preoperative evaluation of tumor margins, allowing optimal therapy.

Materials and Methods

The study was conducted in accordance with the guidelines of local ethics committee and after the informed consent was obtained from the patient.

The dermoscopy analysis was performed using images acquired with a videodermoscope (FotoFinder, Teach screen, Germany).

We used a near-infrared reflectance-mode confocal laser scanning microscope (Vivascope1500, Lucid Inc., Rochester, NY, USA) to acquire the RCM images. RCM allows an *in vivo* non-invasive investigation of skin structure by performing horizontal optical sections of cutaneous tissue at different levels, at a depth of up to 200–350 μm [24]. The optical resolution is similar to classical histological examination and the power of laser source is lower than 30 mW on the skin surface allowing a non-invasive detailed investigation of cutaneous tissue [19, 25, 26]. The frame rate of nine frames per second enables real-time assessment of skin structures and analysis of dynamic phenomena that occur in cutaneous tissue, such as the blood flow through dermal vessels [27, 28]. The area of each individual image is 500×500 μm

and by joining a sequence of multiple digital images acquired at a given depth is generated a mosaic of maximum 8×8 mm.

For *in vivo* analysis of the skin lesion, we obtained five mosaics of 6×6 mm to 8×8 mm at five different depths, and in the areas of particular interest, we acquired full-resolution individual images.

For histopathological examination, the excised tissue was fixed in 10% buffered formalin and embedded in paraffin. After routine processing, slides were stained with Hematoxylin–Eosin (HE) and Van Gieson (VG) light microscopy examination was performed using a optical microscope (Nikon Optiphot 2, Nikon Co., Japan), and pictures were acquired with a digital camera (Olympus C8080WZ, Olympus America Inc., Melville, NY, USA).

Results

A 74-year-old Caucasian male, referred to our clinic for a slowly growing nodular lesion, located on the right supraclavicular region, which he had first noticed two years ago. The patient was light-skinned, with no family history of skin cancer but with a history of intense intermittent sun exposure.

Clinically, the lesion was a translucent nodular tumor of approximately 1×1.5 cm in size, irregular contour and telangiectasias on its surface (Figure 1A).

Dermoscopy showed a multilobular structure with irregularly distributed multiple blue-gray globules and dots as well as arborizing or dilated vessels and numerous fine telangiectasias on the surface and in periphery of the tumor (Figure 1B).

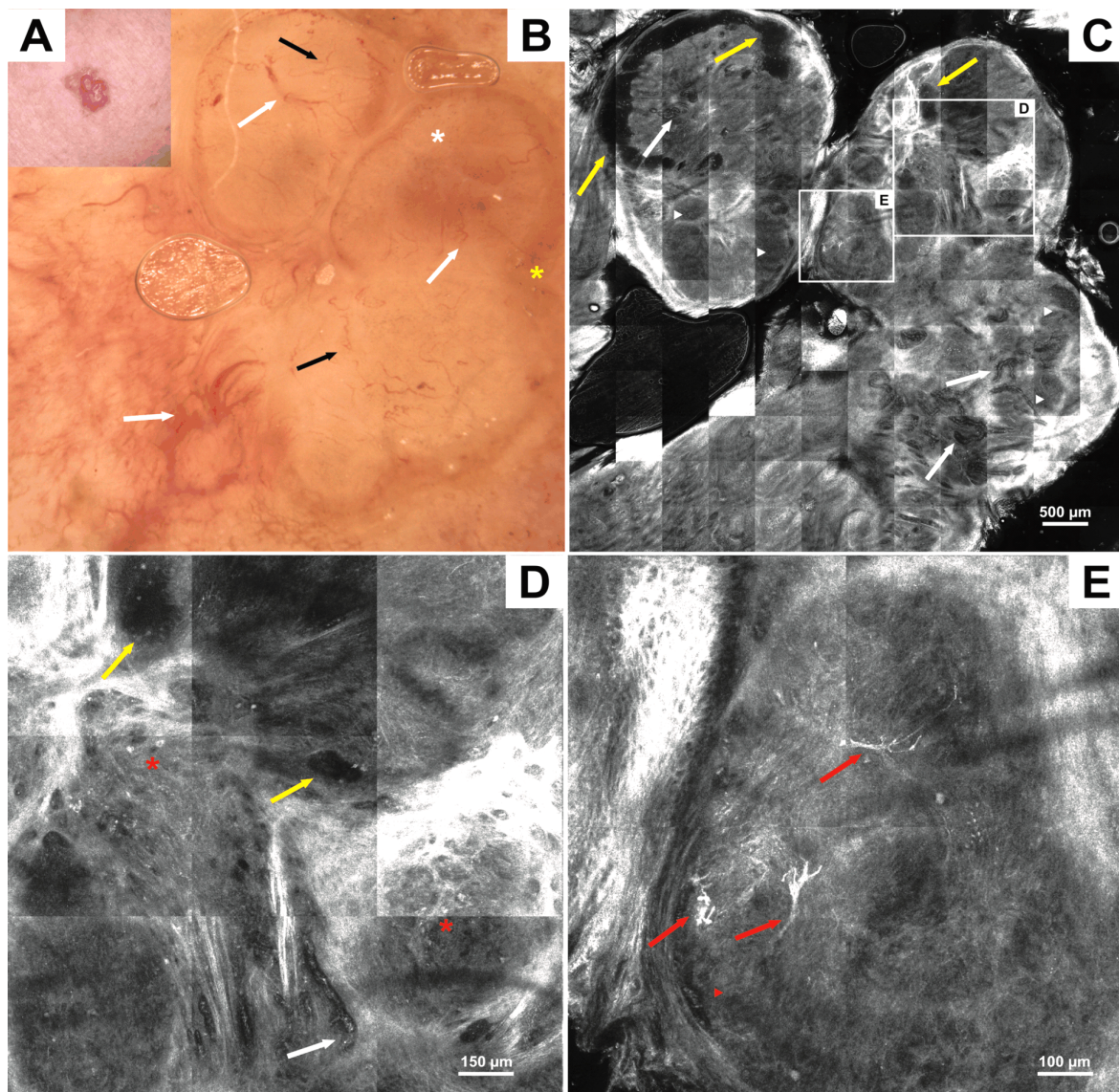


Figure 1 – Correlation between clinical, dermoscopic, and RCM images: (A) Clinical image showing a translucent nodular tumor with irregular contour; (B) Dermoscopy image with multiple blue-gray globules (yellow asterisks) and dots (white asterisks), multiple dilated vessels with tree-like branching (white arrows), and numerous fine telangiectasia (black arrows); (C) RCM mosaic of 6×6 mm showing a multilobular lesion with cauliflower architecture; solid hypo-refractile tumor islands less bright than surrounding stroma (white arrowheads); large non-refractile areas containing bright round-oval structures (yellow arrows); numerous enlarged vessels (white arrows); (D) Detail of RCM image showing non-refractile areas (yellow arrows), bright round-oval structures (red asterisks) and enlarged vessels (white arrow) within a tumor nest; (E) Detail of RCM image showing a tumor island with elongated and polarized nuclei, suggesting a peripheral palisade arrangement (red arrowhead) containing numerous bright thin filaments and large bright dendritic cells (red arrows).

RCM (Figure 1, C–E) revealed a cauliflower architecture created by solid tumoral masses appearing less bright than surrounding stroma. In the center and the periphery of tumoral lobules, large very dark areas with bright structures inside them were present. There were numerous enlarged vessels with increased blood flow in real-time examination inside and in the periphery of the tumoral lobules. At high resolution, in the inner portion

of tumoral masses elongated and polarized nuclei, suggesting a peripheral palisade arrangement were observed. There were also present bright round-oval structures, thin filaments and large dendritic cells.

Clinical, dermoscopy and RCM findings were suggestive of nodular BCC, and after a careful control of lesion borders, the tumor was excised with safety margins of 2 mm.

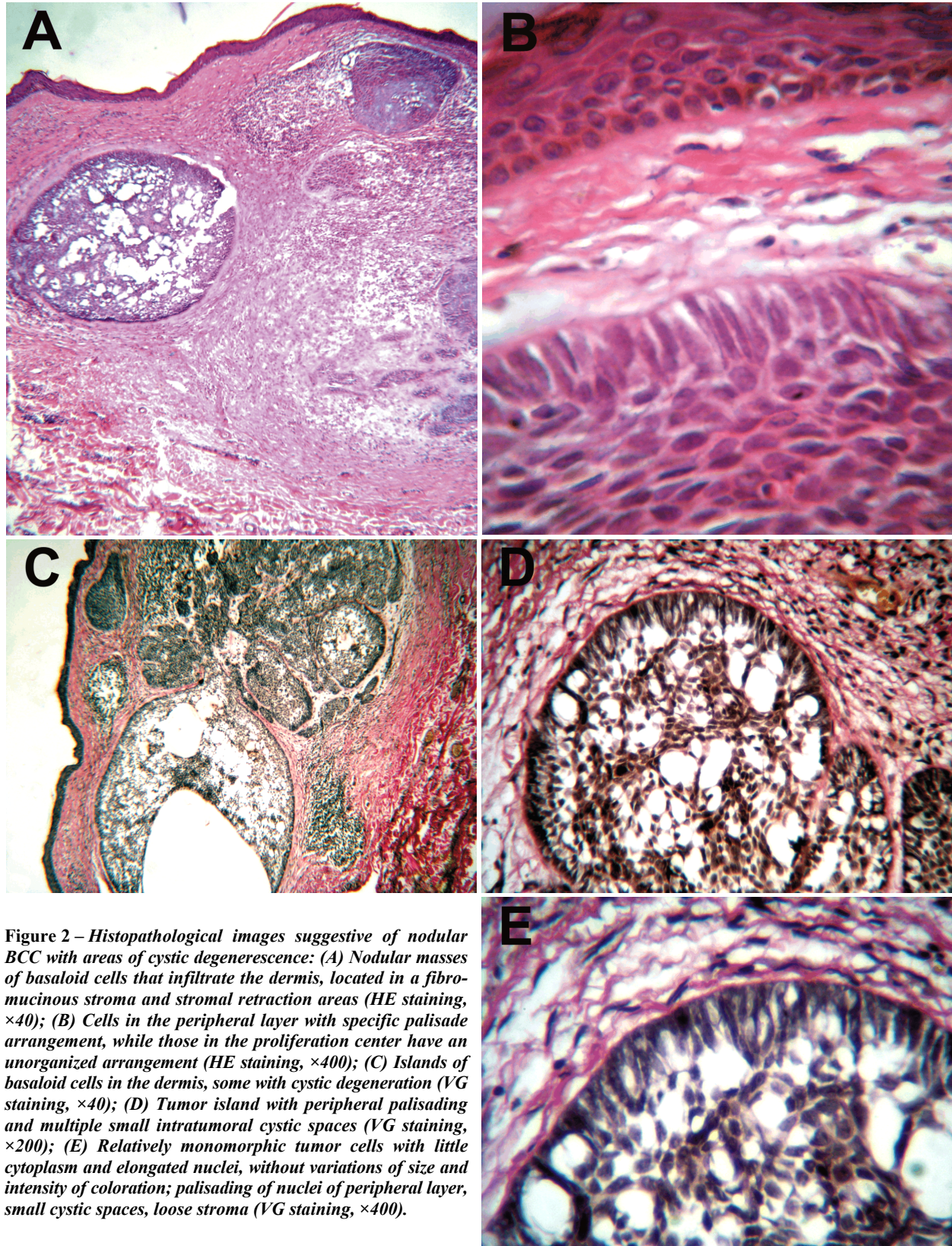


Figure 2 – Histopathological images suggestive of nodular BCC with areas of cystic degeneration: (A) Nodular masses of basaloid cells that infiltrate the dermis, located in a fibromucinous stroma and stromal retraction areas (HE staining, $\times 40$); (B) Cells in the peripheral layer with specific palisade arrangement, while those in the proliferation center have an unorganized arrangement (HE staining, $\times 400$); (C) Islands of basaloid cells in the dermis, some with cystic degeneration (VG staining, $\times 40$); (D) Tumor island with peripheral palisading and multiple small intratumoral cystic spaces (VG staining, $\times 200$); (E) Relatively monomorphic tumor cells with little cytoplasm and elongated nuclei, without variations of size and intensity of coloration; palisading of nuclei of peripheral layer, small cystic spaces, loose stroma (VG staining, $\times 400$).

Histopathological analysis (Figure 2) confirmed the diagnosis of nodular BCC, demonstrating the presence of tumoral masses of relatively monomorphic cells with small amount of cytoplasm, elongated nuclei and specific palisade arrangement in the peripheral layer. There were also present areas of cystic degenerescence, highlighted by intratumoral cystic spaces. The tumor islands were invasive in superior reticular dermis, being surrounded by a fibromucinous stroma with connective fibers arranged parallel to the tumoral surface and areas of stromal retraction from the tumor masses. We also emphasized a moderate peritumoral chronic inflammatory process. The excision margins were clear both laterally and in depth.

Discussion

BCC may display various clinical aspects, frequently has subclinical extension, and through local invasion may induce important tissue damage. Thus, implementation of modern techniques of investigation, such as RCM and dermoscopy is necessary to ensure an accurate diagnosis and a detailed preoperative evaluation of tumor margins. A recent study revealed that dermoscopy investigation of the lesion margins can identify subclinical extension of BCC in a significant number of cases, allowing complete excision of the tumor in 98.5% of cases with safety margins of only 2 mm [16].

Dermoscopic features that can be found in BCC lesions include ulcerations, maple-leaf like structures, spoke-wheel areas, blue-whitish veil, blue-gray ovoid nests, blue-gray globules, arborizing vessels and also multiple erosions, in-focus blue-gray dots and fine vessels, in absence of a pigment network [14, 15, 17, 29]. In the present case, dermoscopy showed the presence of multiple blue-gray globules and dots within a multilobular structure, dilated and arborizing vessels and numerous fine telangiectasias.

The multilobular structure is the dermoscopic appearance of basaloid tumor masses. The blue-gray globules correspond to presence of melanin and melanocytes in tumoral islands [17, 20], while blue-gray dots are a preliminary stage of multiple blue-gray globules, corresponding to clusters of pigment within tumor nests located in upper dermis [4, 17]. Fine telangiectasias are a preliminary stage of arborizing vessels [4, 17, 30], a frequent dermoscopic feature of BCC [29, 31].

RCM is a non-invasive imaging technique that allows the investigation of skin tissue in horizontal plane, similar to dermoscopy [20] and provides images at nearly histological resolution [21, 25, 26]. Thus, RCM characteristics of BCC, previously described by other studies [18, 20, 32, 33] may correlate with both dermoscopy and histopathological features.

In our study, RCM showed the presence of solid tumoral nests less bright than surrounding stroma with elongated and polarized nuclei with peripheral palisade arrangement, which correspond to histological aspect of basaloid cells tumor lobules. The large non-refractile areas observed in the center and the periphery of tumoral masses correlate with areas of cystic degenerescence that appear in classic histological examination as large cavities filled with amorphous debris and degenerated epithelial cells. The increased number and diameter of blood vessels

correlate with arborizing vessels and the numerous fine telangiectasias in dermoscopy and may suggest a process of neoplastic angiogenesis [23], which may give rise to abnormal fenestrated capillaries in the peritumoral stroma [34]. The bright, round-oval structures that were observed in the tumor nests correlate with blue-gray granules and dots on dermoscopy and correspond to melanin-rich melanophages [20, 21]. The presence of highly refractive thin filaments and large dendritic cells in tumor islands correspond to melanocytes or Langerhans cells on histology [20, 35].

In vivo evaluation, preserving an unaltered tissue architecture, investigation in horizontal section of the skin and image acquisition from large tissue areas specific to dermoscopy and RCM offers a different perspective on micromorphological structure of the skin, and allows detection of structural details, such as the presence of pigment clusters, melanin-rich melanophages and Langerhans cells within tumor islands, which may not be noticed during routine histological examination. It is also facilitated the evaluation of vascular changes accompanying the cutaneous neoplastic process.

Conclusions

RCM evaluation allows the preoperative highlighting of histological characteristics of nodular BCC with areas of cystic degeneration and the use of RCM in combination with dermoscopy enables *in vivo* determination of tumor margins, allowing the optimization of surgical treatment, with best aesthetic and functional results.

Acknowledgments

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Corresponding author

Daniel Boda, MD, PhD, Dermatology Research Laboratory, “Carol Davila” University of Medicine and Pharmacy, 22–24 Grigore Manolescu Street, Sector 1, 0111234 Bucharest, Romania; Phone +40757–079 117, Fax +4021–222 13 10, e-mail: daniel.boda@yahoo.com

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