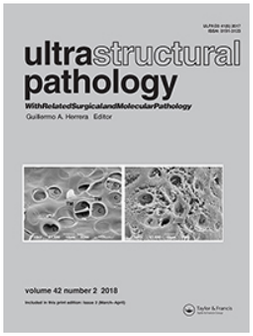

Ultrastructural Pathology

ISSN: 0191-3123 (Print) 1521-0758 (Online) Journal homepage: http://www.tandfonline.com/loi/iusp20

Ultrastructure of colorectal adenocarcinoma and peritumoral tissue in untreated patients

Hector L. Osorio, Hector J. Finol, L. Roschman Gonzalez & Carlos E. Sardiñas

To cite this article: Hector L. Osorio, Hector J. Finol, L. Roschman Gonzalez & Carlos E. Sardiñas (2018) Ultrastructure of colorectal adenocarcinoma and peritumoral tissue in untreated patients, Ultrastructural Pathology, 42:2, 81-90, DOI: 10.1080/01913123.2017.1422064

To link to this article: https://doi.org/10.1080/01913123.2017.1422064

Published online: 08 Feb 2018.

ULTRASTRUCTURAL PATHOLOGY
2018, VOL. 42, NO. 2, 81–90
https://doi.org/10.1080/01913123.2017.1422064

Taylor & Francis
Taylor & Francis Group

CLINICAL RESEARCH

Ultrastructure of colorectal adenocarcinoma and peritumoral tissue in untreated patients

Hector L. Osorio [a], Hector J. Finol[b], L. Roschman Gonzalez[b], and Carlos E. Sardiñas[c]

[a]Laboratory for Cellular and Molecular Pathology, Venezuelan Institute for Scientific Research, Altos de Pipe, Miranda, Distrito Capital, Venezuela; [b]Center for Electron Microscopy, Faculty of Science, Central University of Venezuela, Caracas, Venezuela; [c]Coloproctology Unit, University Hospital of Caracas, Central University of Venezuela, Caracas, Venezuela

ABSTRACT

In this study, we describe, compare, and discuss several subcellular alterations found in Colorectal Adenocarcinoma and peritumoral tissue using transmission electron microscopy, morphometry, and statistical analysis. Tissue samples from anterior resections were collected from patients diagnosed with Colorectal Adenocarcinoma in the University Hospital of Caracas. Samples were processed according to the typical protocol for their observation through transmission electron microscopy. The resulting images were analyzed using specialized software for the collection of morphometric data. Several anomalies were common for both tissues, including but not limited to, rough endoplasmic reticulum and mitochondrial swelling, nuclear invagination, nuclear enlargement, and cellular swelling. In general, alterations within the tumor were more frequent and intense. Extensive organellar degradation and other evidences of cellular damage seemed to extend past the edge of the tumor into the peritumoral tissue. There seems to be a clear process of lateral cancerization present in the peritumoral area. The tissue layers composed of smooth muscle cells, probably due to their structural features, may allow greater diffusion of harmful substances produced by the tumor. A more in-depth analysis of peritumoral tissue considering organellar damage and morphometric data may provide relevant insight about the changing microenvironment promoted by the close proximity of a tumor.

ARTICLE HISTORY
Received 4 April 2017
Accepted 22 December 2017
Published online 9 February 2018

KEYWORDS
Cancerization; colorectal; peritumoral; tumor; ultrastructure

Introduction

The study of ultrastructural alterations in tumoral and peritumoral tissue from colon adenocarcinoma (CAC) has been limited so far. Only certain aspects of cell alteration within the tumor are usually taken into consideration and in the case of peritumoral tissue, very few studies even address it, even when proper evaluation of the tumor surroundings is paramount for the effective surgical treatment of patients.

The usefulness of certain ultrastructural features such as microvilli and glycocaliceal bodies in the fine process of distinguishing between CAC and other carcinomas has been suggested in the past.[1] Some researchers have even associated morphological alterations of cancer cell organelles such as mitochondria with carcinogenesis,[2] in some cases even comparing those findings with mitochondriopathies.[3] Therefore, it is possible that other ultrastructural elements could be harnessed for the evaluation of this particular type of cancer.

Ultrastructural comparisons between tumoral and peritumoral tissues are not very common; however, some examples include (a) studies on the characterization of ultrastructural features of apical, lateral, and basal domains of colon cancer cells at different tumor levels that were performed to determine whether there is an ultrastructural continuum from organized cells to completely disorganized cells at the advancing edge of tumor,[4] (b) descriptions of peritumoral ultrastructure in patients with rectal cancer treated with chemotherapy and radiotherapy,[5] and (c) a recent study performed by our team where we found three forms of cell death (apoptosis, necrosis, and autophagy) differentially distributed between the adenocarcinoma and its surrounding tissue.[6]

In this paper, we use transmission electron microscopy, image processing software, and morphometry

CONTACT Hector J. Finol hector.finol@ciens.ucv.ve Center for Electron Microscopy, Faculty of Science, Central University of Venezuela, Caracas, Venezuela

to describe ultrastructural alterations in tumoral and peritumoral tissue from a small sample of patients diagnosed with CAC and treated only with anterior resection. We wish to clarify that none of the subcellular anomalies to be presented in this study are novel and in fact have been frequently described in the past; however, they remain underexplored within the context of cancer when they could potentially provide patient-relevant information. Therefore, it is our hope to encourage further research into the subcellular abnormalities found in this particular manifestation of cancer.

Materials and methods

Patients

Patients with CAC included in this study were treated with anterior resection in the coloproctology unit at the University Hospital of Caracas (UHC). The project was approved by the ethics committee of the hospital and the subjects gave their written consent. Colon biopsies were taken from the tumoral and peritumoral areas (first 5 cm from the tumor margin) of four male patients (aged between 45 and 75 years old) diagnosed with CAC in stages C and D according to Duke´s modified staging system.[7]

Sample processing

Tumor and peritumoral samples were collected from patients at the moment of anterior resection and then immediately submerged in cold (2°C) fixative to avoid tissue degradation that could interfere with results. All manipulations of unfixed tissue were carried out by medical staff under sterile conditions. Manipulation of fixed samples was performed by experienced laboratory personnel using sterile tools and following the standard protocol for transmission electron microscopy.

Samples were fixed with 1% Karnovsky fixative in a 320 mOsmol Millonig buffer, pH 7.4, post-fixed in 1% OsO_4, dehydrated in ethanol and embedded in Embed-812 resin (EMS, Hatfield, PA, USA). Ultrathin sections stained with uranyl acetate and lead citrate were observed in a JEOL JEM-1011 transmission electron microscope operated at 80 kV.

Morphometry

Morphometric data were collected from tumor and peritumoral tissue. Since the purpose of this study is to describe overall changes in these two areas, we did not categorize data by cell type; we simply gather information from equal proportions of epithelial and mesenchymal cells and exclude any infiltrating cells to then classify measurements by the area in which they were obtained.

All cells represented in this study both in pictures and statistics were either located at the mucosa (in the case of peritumoral tissue) or at the middle surface of the tumor (in the case of tumoral tissue were the degree of dysplasia prevents proper layer visualization).

Measurements of subcellular structures were collected using ImageTool for Windows.[8]

Statistics

Statistical analysis was performed by means of the STATISTICA Software.[9] The data were expressed as values of area (mitochondria and nuclear envelope) or length/thickness (rough endoplasmic reticulum cisternae, RERC). Descriptive statistics were used in the form of graphs to show the differences of data distribution between the tumoral and peritumoral areas. The Student's t-test ($p = 0.05$) (for nuclear area, NA and mitochondrial area, MA) and the Mann–Whitney U test ($p = 0.05$) (for thickness of rough endoplasmic reticulum cisternae, TRERC) were used as a way to establish the existence of statistically significant differences between the measurements taken from the two sampled tissue areas.

Non-parametric statistics were used only in cases where data distribution proved to be non-normal.

Results

Ultrastructure

Different ultrastructural alterations were found in epithelial cells of CAC (tumor). Initially changes were seen in mitochondrial morphology (Figure 1(a,b)). On the contrary, rough endoplasmic reticulum (RER) looked normal (Figure 1(a)). Furthermore, in addition to cells with normal looking RER, other cells appeared with RER alterations consisting of swollen cisternae (Figure 1(b)) and rests of RER participating in the formation of autophagosomes (Figure 1(c)). In relation to the Golgi apparatus,

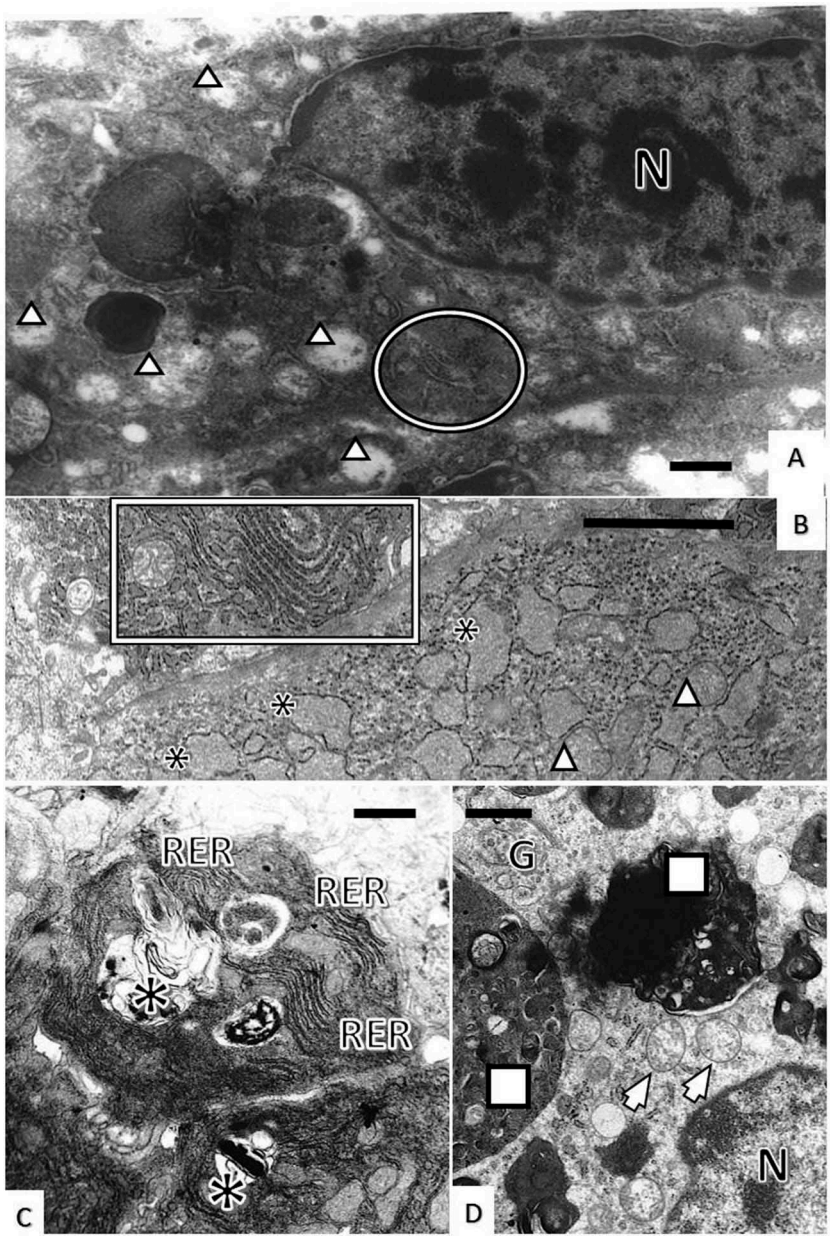

Figure 1. a) Section of tumor cell. Nucleus (N), rough endoplasmic reticulum (circle), and swollen mitochondria (triangles) (bar: 1 µm). b) Section of tumor cell. One cell exhibits swollen RERC (asterisks) and mitochondria (triangles). The other shows normal looking RERC and swollen mitochondria (rectangle) (bar: 2 µm). c) Section of tumor cells. Rough endoplasmic reticulum cisternae (RER) participate in the formation of autophagosomes (asterisks) (bar: 1 µm). d) In this section, see Golgi apparatus (G), autophagosomes (squares), swollen mitochondria (arrowheads) and nucleus (N) (bar: 1 µm).

there was a significant cisternae reduction and its presence was observed in areas with autophagosomes (Figure 1(d)). In these cells, nucleus exhibited a normal ultrastructure (Figure 1(a, d)). Nevertheless, other cells showed nuclei with deep invaginations and nucleoli with a very electron dense aspect (Figure 3(c)). In advanced stages of degeneration and necrosis, nuclei presented heterochromatin alteration and loss of nuclear envelope with nuclear matrix extraction (Figure 2(a,b)). Mitochondrial and RER swelling was also seen in the prometaphase of some dividing cells (Figure 2(c)), In this case, a chromosome still covered with rests of nuclear envelope was found. In addition, loss of intercellular desmosomes was detected (Figure 2(d)),

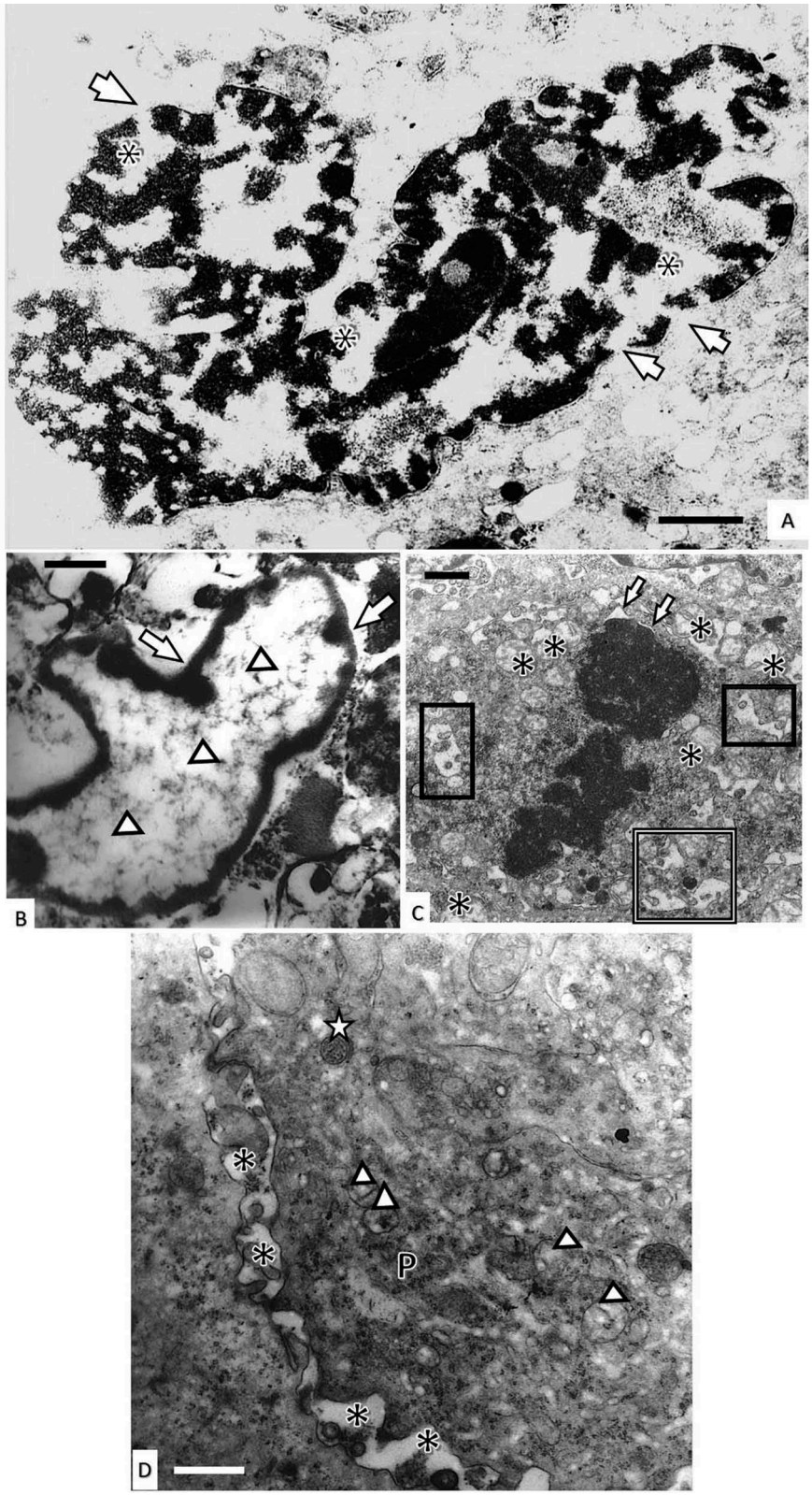

Figure 2. a) In this section, see heterochromatin (asterisks) and nuclear envelope (arrowheads) loss (bar: 1 µm). b) In this section, nuclear matrix extraction (triangle) and heterochromatin condensed and devoid of nuclear envelope (arrowheads) (bar: 0.5 µm). c) Section of cell in prometaphase. Nuclear envelope rests (arrows), swollen mitochondria (asterisks) and RER (rectangles) are seen (bar: 1 µm). d) In this section, swollen mitochondria (triangles), intercellular spaces (asterisks), polysomes (P) and a multivesicular body (star) (bar: 1 µm).

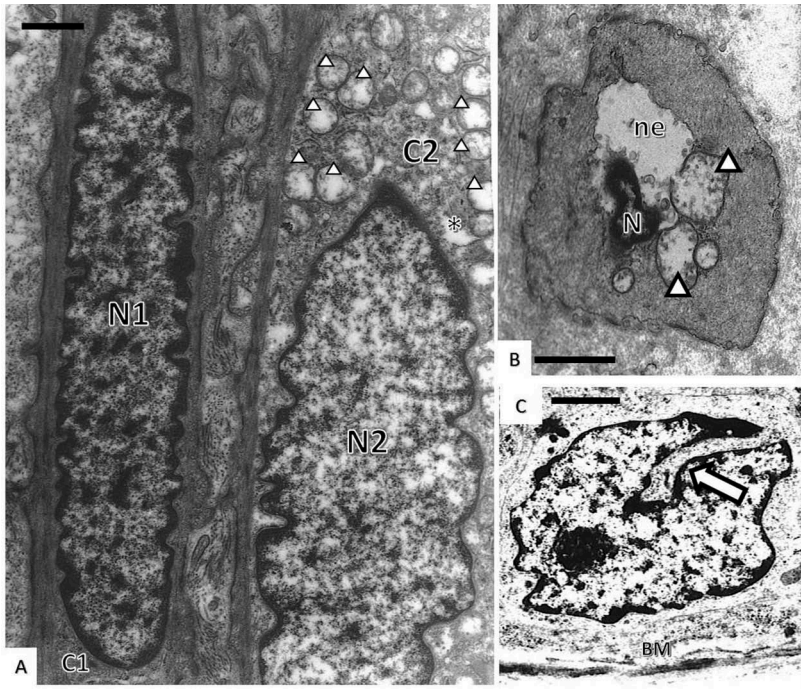

Figure 3. a) Two smooth muscle cells side by side, one of the cells with folded nuclear envelope showed a less electron-dense nuclear matrix (N2) and cytoplasm (C2) than the other (N1). The cell with N2 nucleus also presents swollen mitochondria (triangles) and RERC (asterisk) (bar: 1 µm). b) Oblique section of a smooth muscle cell containing a nucleus sectioned at the periphery (N) and with a swollen nuclear envelope (ne). Also, there are some examples of swollen mitochondria (triangle) (bar: 1 µm). c) Section of epithelial cell with deep invagination of the nuclear envelope (arrow), basal membrane is visible (BM) (bar: 0.5 µm).

the altered cells also presented mitochondrial swelling, abundant polysomes and the disappearance of RER. Note also the presence of multivesicular bodies.

In the peritumoral area, smooth muscle cells presented swelling of mitochondria and nuclear envelope (Figure 3(a,b)). Dense bodies were rarely seen in myofilament associations but dense plaques were frequently observed along the surface of cells. Some epithelial cells with severe invagination of nuclear envelope were also detected (Figure 3(c)).

Statistics

For MA, the tumoral tissue contained many extreme values (Figure 4). There is an obvious difference between the two zones in which sampled tumoral mitochondria have (in general) a larger area than their peritumoral counterparts ($p < 0.05$) (Table 1), indicating that larger/swollen mitochondria are more frequent in the tumoral tissue; however, there are a few examples of swollen mitochondria at the peritumoral zone, suggesting that in this zone mitochondrial swelling is less frequent and intense but still present.

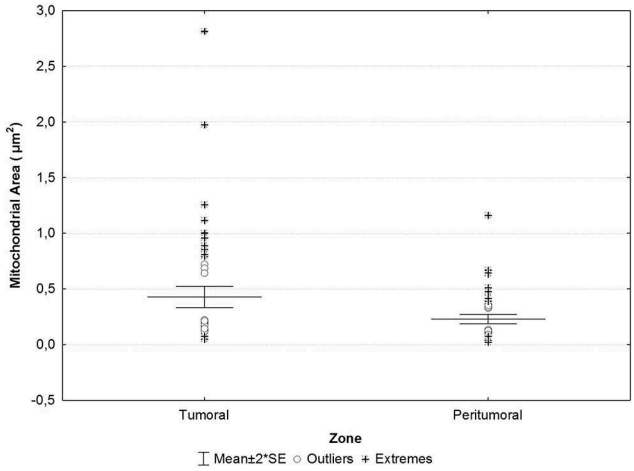

Figure 4. Mitochondrial area on tumoral and peritumoral tissues. *X*-axis: sampled zone and *Y*-axis: mitochondrial area (µm^2). Line: mean. Whiskers: mean ± 2 standard errors (SE). Outliers: values at a distance equal or greater than 3 SE from the mean. Extremes: values at a distance equal or greater than 6 SE from the mean. $p < 0.05$: Statistical comparison.

The measurements of NA revealed a nuance difference between the tumoral and peritumoral zones (Figure 5). In this case, the tumoral zone contained the smaller values with few extremes and outliers;

Table 1. Statistical results for measured parameters in tumor and peritumoral tissue.

Morphometric parameters	Tumor/peritumoral
NA	$p < 0.05$
MA	$p < 0.05$
TRERC	$p < 0.05$

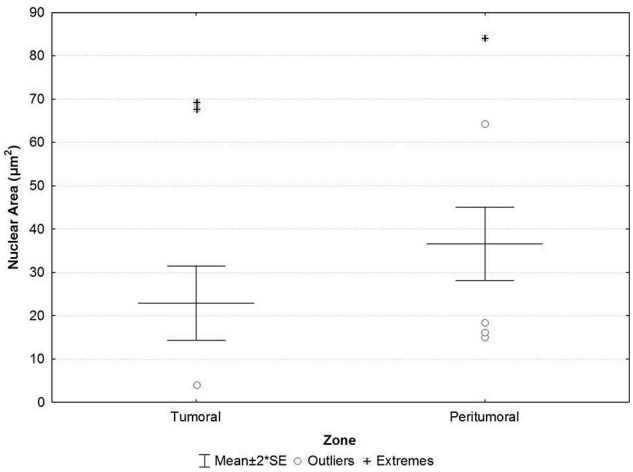

Figure 5. Nuclear area on tumoral and peritumoral tissues. *X*-axis: sampled zone and *Y*-axis: nuclear area (µm^2). Line: mean. Whiskers: mean ± 2 standard errors (SE). Outliers: values at a distance equal or greater than 3 SE from the mean. Extremes: values at a distance equal or greater than 6 SE from the mean. $p < 0.05$: Statistical comparison.

meanwhile, the peritumoral area contained larger values with several outliers and extremes indicating a disparity that was later confirmed with a parametrical test ($p < 0.05$) (Table 1). These findings suggest a greater amount of nuclei with invaginations in their envelope within the tumoral zone and a lesser amount in the peritumoral area with a few examples of enlarged nuclei.

The comparison of TRERC between the two zones (Figure 6) revealed a very marked difference between them ($p < 0.05$) (Table 1). The tumoral tissue contained the higher values with several accentuated outliers and extremes; meanwhile, the peritumoral tissue contained lower and more homogenous values of thickness with less-accentuated outliers and extremes. This suggests that in the tumor swollen RERC are much more frequent and the intensity of this alteration is much greater. Moreover, it indicates that many RERC can be found at different stages of swelling.

Discussion

We encountered several morphological alterations within the tumor and in the peritumoral tissue. Structural changes of RER, mitochondria, and nuclear envelope appear to be among the most common examples of subcellular defects.

Organellar alterations have been reported in the past in many different circumstances. One interesting example is the one reported by Meng and Lui in which mice exposed to SO_2-manifested

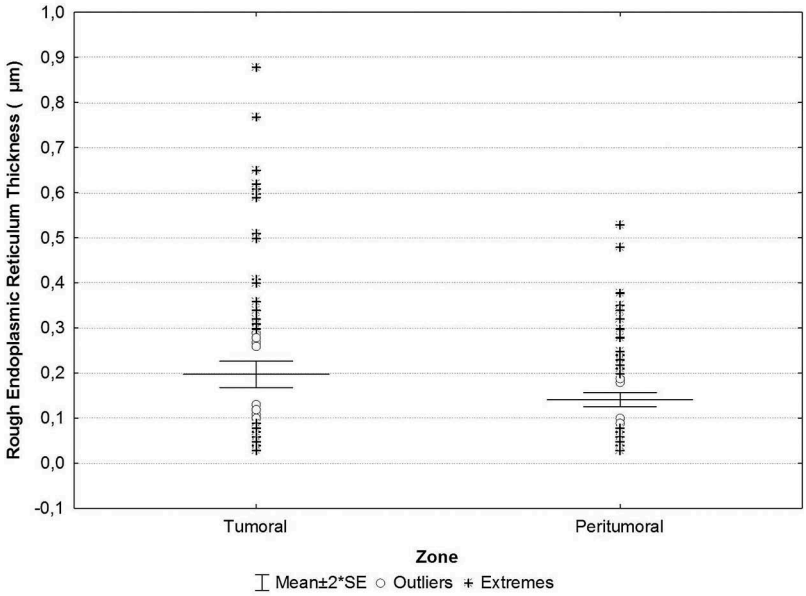

Figure 6. Thickness of rough endoplasmic reticulum cisternae (RERC) on tumoral and peritumoral tissues. *Y*-axis: sampled zone and *Y*-axis: RERC thickness (µm). Line: mean. Whiskers: mean ± 2 standard errors (SE). Outliers: values at a distance equal or greater than 3 SE from the mean. Extremes: values at a distance equal or greater than 6 SE from the mean. $p < 0.05$: Statistical comparison.

systemic abnormalities with very similar sub-cellular alterations to the ones observed by our team in colorectal adenocarcinoma.[10] Although morphological changes in organelles are not an exclusive feature of cancer tissues, they can be a good indicator of exposure to an stressful environment.

Many tumoral and peritumoral mitochondria were found to be swollen. Most exhibited severe degradation of their internal structure and in some cases even the external membrane was found to be ruptured. Morphologically abnormal mitochondria are a common feature in many different types of cancers and cancer cell lines.[2,11,12]

Abnormal tumor mitochondria have been associated with the process of aerobic glycolysis (also known as the Warburg effect), which involves inhibition of cellular respiration from impaired oxidative phosphorylation and development of resistance to chemotherapy.[13] Some researchers have implicated the mitochondrial permeability transition pore (PTP) and other proteins on the mitochondrial membrane such as Bcl2 to be important elements in the development of the swelling.[14]

We observed mitochondrial swelling in cells from tumor stroma and in smooth muscle cells from the peritumoral area. At this point, it seems important to state that mitochondrial swelling has been related to the production of mutagenic agents like reactive oxygen species.[14] Alterations in respiratory activity are therefore accompanied by somatic DNA mutations. Moreover, the prevalence of inherited mitochondrial DNA polymorphisms in cancer patients has been reported as a relevant element in cancer development.[3]

After revising the data, we were able to find that mitochondrial swelling is much more frequent and intense within the tumor. This creates a kind of paradox since many of the proposed mechanisms for mitochondrial swelling are also related to the process of apoptosis through the release of cytochrome c and this is the one event that should be difficult to find in neoplastic tissue.

The aforementioned paradox can be resolved with the discovery of a mechanism that prevents cytochrome c-mediated apoptosis (CycMA). Vaughn et al. reported a possible mechanism common for neurons and cancer cells in which CycMA is inhibited under the conditions of glycolytic metabolism and high levels of intracellular glutathione,[15] allowing the presence of damaged or ruptured mitochondria without initiation of apoptosis.

Mitochondrial and RERC swelling were found to display similar patterns between the two sampled zones. RERC dilation/swelling is a particular alteration that is not frequently addressed within the context of cancer. The most common example of cisternae dilation in cancer comes from ultrastructural studies performed on myeloma in which it is attributed to the extreme biosynthetic process associated with the production of Immunoglobulins.[16]

Swollen mitochondria and RERC have been observed in sheep with CAC.[17] In the past, alterations of RERC have not been listed as diagnostically useful organellar changes for the characterization of colorectal adenocarcinoma.[18] RER dilated cisternae have been described as a feature of neoplasms composed of fibroblast-like cells.[19] According to Thomopoulus, RER swelling is not always a sign of cell necrosis.[20] It is interesting that the formation of autophagosomes from RER was described in a case of melanosis coli and in a child who had an islet cell adenoma of pancreas. Ghadially admitted that formation of autophagosomes could originate from de Golgi region.[21]

Cisternae dilation in other pathological contexts have been associated with disorders of the secretory mechanism of the cell, accumulation of misfolded proteins, and accumulation of large viral proteins.[22,23] In cancer, as far as we could tell, there is not a single clear mechanism for the dilation of the reticulum, but the presence of a hypoxic environment and coexpression of Bak and BCL-Xl have been linked as relevant players in the process.[16,24]

We were also able to detect several different nuclear abnormalities. Irregular nuclear contours are commonly observed in human neoplasms.[25] They can be represented by deep cytoplasmic invaginations resulting in pleomorphic nuclei as in the present case or they can consist of shallow deformations of the nucleus by the pressure of the cytoplasm when sectioned, allowing the formation of nuclear pseudo-inclusions.[18,19]

Sometimes the nucleus can be so extensively segmented and beset by invaginations that it may assume an almost sponge-like aspect.[25] Marginal nucleoli have been reported in adenocarcinomas and in non-neoplastic cells with very elevated synthetic activity.[19] In this case, we did not observe such nucleoli. On the contrary, compact electron-dense nucleoli as they have been described in carcinomas were seen in the present work.[19,25] Necrotic nuclei have been described as containing blocks of heterochromatin and poorly preserved nuclear envelope.[18] In addition to necrosis, autophagy and apoptosis are found in human adenocarcinoma as a form of cell death.[6]

Many different molecular events have been associated with a variety of nuclear aberrations. Quantitative and qualitative changes of lamin, gene silencing, and interactions between inner nuclear membrane proteins and extracellular matrix are among the most common.[26] Altered lamin expression can change nuclear shape and has been identified as a common feature in gastrointestinal cancers[27]; therefore, this particular anomaly might play a part on our cases of nuclear invagination.

Several examples of nuclear enlargement were found in the peritumoral zone. Enlarged nuclei are known as "early warning signs." This alteration is very common at the stages of dysplasia or carcinoma *in situ*, when the probability of metastasis is low.[28] The same nuclear anomaly is also observed in tissues treated with carcinogens at low doses. It is also one of the first visible manifestations of cellular damage in these cases.[29]

Swelling of the perinuclear space was found in cells from the peritumoral tissue. Enlargement of the perinuclear space has been reported as a common alteration in cancer, most notably, as a characteristic of small cell lung cancer.[30] It is believed that this can be caused by disturbances in the composition of the nuclear envelope.[31]

Structural remodeling of the nucleus within the context of cancer cells can lead to a more invasive phenotype. This allows greater malleability and deformability of the nucleus which favors cancer progression and invasion. In colon cancer, these alterations are usually associated with changes in the expression of lamin A or C and to an increased risk of cancer recurrence.[31]

A decrease in the number of desmosomes and loss of structural cohesion in malignant tumors and during carcinogenesis has been reported.[21] We observed reduced number of desmosomes during our study. Such a reduction was described in cell culture of human colon carcinoma cells.[32] That study showed that *N,N*-dimethylformamide, which induces differentiation of human colonic carcinoma, leads to a sixfold increase in the number of desmosomes. In sheep adenocarcinoma of small intestine desmosome reduction has not been observed.[17]

There are very few studies that use MA, NA, and/or TRERC as morphometric parameters for the evaluation of a particular tissue. MA has been used either directly or indirectly and together with other morphometric parameters like cytoplasmic area to characterize a particular sub-population of cancer cells or to evaluate the extent of mitochondrial damage caused by medication.[33–35]

NA has also been used as a way to characterize a sub-population of cancer cells. During the period, it was also considered as an independent parameter with potential value for patient prognosis; however, this approach has remained underdeveloped so far.[36,37]

In the case of TRERC, we were not able to find a single study where this parameter was used directly or indirectly. Many studies describe RER alteration, most of which include cisternae swelling; however, the morphometric approach seems to be absent in the literature.

Finol et al. (1994) reported very similar alterations to the ones observed by our team in nervous and muscular tissue adjacent to a fibrous histiocytoma. They proposed that one possible explanation for the presence of peritumoral anomalies could be a local manifestation of the "paraneoplastic phenomenon," a capacity of malign tumors to cause damage in non-invaded organs. Our findings to this point seem to agree with theirs.[38]

If we take all the evidence into consideration, and include the fact that many of the ultrastructural alterations found in peritumoral tissue seem to mirror the ones found in the tumor, it becomes noticeable that the pattern of morphological abnormalities and spatial distribution suggests an event of "lateral cancerization" in which there is a progressive affectation of cells adjacent to the tumor that increases the probability of carcinogenesis within the affected field of cells,

meaning that although the peritumoral tissues cannot be considered as cancerous, with time they can become more prone to manifest neoplastic growth. It has been reported that in colorectal cancer this event is much easier to observe than in other organs due to the continuity of the epithelial tissue.[40]

Lateral cancerization and other modes of field cancerization associated with tumors with evidence of decrease in their number of caveolae have been linked with the propagation and amplification of stromal oxidative stress related to the production of reactive oxygen species and nitrous oxide in a "contagious" manner, which allows the manifestation of genetic, biochemical, and morphological alterations in the peritumoral tissue.[40]

At the peritumoral zone, we observed most of the abnormalities in smooth muscle cells. As shown in smooth muscle cells from peritumoral areas of rectal cancer from patients treated and non-treated with chemotherapy and radiotherapy,[5] in our case disorganization of contractile system, swelling of mitochondria, and necrosis were present. This could indicate that tissue layers composed of smooth muscle in this organ, due to their less intimate connection between cells, have a greater capacity to allow diffusivity, permitting increased dispersion of harmful substances. More research on this topic is needed to reveal the mechanisms responsible for peritumoral alteration as well as the magnitude of their impact on the tissue and the precise spatial distribution of their influence.

Conclusion

- Tissue layers composed of smooth muscle cells in the colorectal area seem to allow greater diffusion of harmful substances produced on the tumor.
- Organelle abnormalities are common for both tumor and peritumoral tissue.
- The adenocarcinoma and the peritumoral tissue display distinct patterns of morphometric parameters like MA, NA, and TRERC.

Declaration of interest

The authors state that the content of this article is original and has not been published previously nor has been submitted for its publication in another journal. The authors are aware that not disclosing if the submitted manuscript has already been send for publication is considered a severe lack of scientific ethics.

The authors have taken into account the proper ethical guidelines. Through this statement we declare that:

(1) The procedures followed during our study conform to the guidelines of the University
(2) Hospital of Caracas's ethics committee, the World Medical Association and the Helsinki Declaration.
(3) We guarantee the right to privacy and confidentiality of all patients involved with this study.
(4) We have obtained informed consent from all patients involved in this study.

There are no conflicts of interests.

ORCID

Hector L. Osorio http://orcid.org/0000-0002-9831-0474

References

1. Hickey W, Seiler M. Ultrastructural markers of colonic adenocarcinoma. *Cancer*. 1981;47:140–145.
2. Arismendi-Morillo G. Electron microscopy morphology of the mitochondrial network in human cancer. *Int J Biochem Cell Biol*. 2009;41:2062–2068.
3. Czarnecka A, Czarnecki J, Kukwa W, et al. Molecular oncology focus - Is carcinogenesis a 'mitochondriopathy'? *J Biomed Sci*. 2010;17:31–38.
4. Lloreta-Trull J. Intercellular junctions, apical differentiation and infiltrative features in colon cancer: an ultrastructural study. *Ultrastruct Pathol*. 2001;25:289–294.
5. Sierra-Añez S, Finol H, Roschman-Gonzlez A, et al. Ultraestructura peritumoral en pacientes con cáncer de recto y tratados con quimioterapia y radioterapia. *Acta Microsc*. 2013;22:210–217.
6. Osorio-Vega H, Finol H, Roschman-Gonzalez A, et al. Colon adenocarcinoma and cell death types. *Acta Microsc*. 2016;25:65–70.
7. Gunderson L, Sosin H. Areas of failure found at reoperation (second or symptomatic look) following "curative surgery" for adenocarcinoma of the rectum. Clinopathologic correlation and implications for adjuvant therapy. *Cancer*. 1974;34:1278–1292.
8. Wilcox C, Dove S, McDavid W, et al. *UTHSCSA Image Tool for Windows, Version 2.01*. San Antonio, TX: University of Texas Health Science Center; 2000.
9. StatSoft Inc. *STATISTICA (Data Analysis Software System), Version 6*. Tulsa, USA; 2001:Vol. 150
10. Meng Z, Lui Y. Cell morphological ultrastructural changes in various organs from mice exposed by

inhalation to sulfur dioxide. *Inhal Toxicol*. 2007;19:543–551.
11. Chiche J, Rouleau M, Gounon P, et al. Hypoxic enlarged mitochondria protect cancer cells from apoptotic stimuli. *J Cell Physio*. 2009;222:648–657.
12. Giang A, Raymond T, Brookes P, et al. Mitochondrial dysfunction and permeability transition in osteosarcoma cells showing the Warburg effect. *J Biol Chem*. 2013;288:33303–33311.
13. Seyfried T, Shelton L. Cancer as a metabolic disease. *Nutr Metab*. 2010;7:7.
14. Batandier C, Leverne X, Fontaine E. Opening of the mitochondrial permeability transition pore induces reactive oxygen species production at the level of the respiratory chain complex I. *J Biol Chem*. 2004;279:17197–17204.
15. Vaughn A, Deshmukh M. Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. *Nat Cell Biol*. 2008;10:1477–1483.
16. Lin J, Walter P, Yen B. Endoplasmic reticulum stress in disease pathogenesis. *Annu Rev Pathol*. 2008;3:399–425.
17. Ross A, Day W. An ultrastructural study of adenocarcinoma of the small intestine in sheep. *Vet Pathol*. 1985;22:552–560.
18. Eyden B. *Organelles in Tumor Diagnosis. An Ultrastructural Atlas*. New York, USA: Igaku Shoin; 1996.
19. Erlandson R. *Diagnostic Transmission Electron Microscopy of Tumors*. New York, USA: Raven Press; 1994.
20. Thomopoulus G. The RER swelling is not always a sign of cell degeneration. *J Submicrosc Cytol*. 1987;19:57–62.
21. Ghadially F. *Ultrastructural Pathology of the Cell and Matrix*. Boston, USA: Butterwords - Hinemann; 1997
22. Ns-I TO, Konishi H, Makino T, et al. Chronic stress elicits prolonged activation of alpha-MSH secretion and subsequent degeneration of melanotroph. *J Neurochem*. 2009;109:1389–1399.
23. Umebayashi K, Hirata A, Fukuda R, et al. Accumulation of misfolded protein aggregates leads to the formation of Russell body-like dilated endoplasmic reticulum in yeast. *Yeast*. 1997;13:1009–1020.
24. Klee M, Pimentel-Muiños F. Bcl-XL specifically activates Bak to induce swelling and restructuring of the endoplasmic reticulum. *J Cell Biol*. 2005;168:723–734.
25. Ghadially F. *Diagnostic Electron Microscopy of Tumors*. London, UK: Butterwords-Hinemann; 1980.
26. Dey P. Cancer nucleus: morphology and beyond. *Diagn Cytopathol*. 2009;38:382–390.
27. Moss S, Krivosheyev V, de Souza A, et al. Decreased and aberrant nuclear lamin expression in gastrointestinal tract neoplasms. *Gut*. 1999;45:723–729.
28. Backman V, Wallace M, Perelman L, et al. Detection of preinvasive cancer cells. *Nature*. 2000;406:35–36.
29. Clawson G, Blankenship L, Rhame J, et al. Nuclear enlargement induced by Hepatocarcinomas alters ploidy. *Cancer Res*. 1992;52:1304–1308.
30. Zink D, Fischer A, Nickerson J. Nuclear structure in cancer cells. *Nat Rev Cancer*. 2004;4:677–687.
31. Denais C, Lammerding J. *Nuclear Mechanics in Cancer*. New York, USA: Springer New York; 2014
32. Christensen T, Burke B, Dexter D, et al. Ultrastructural evidence of dimethylformamide - induced differentiation of cultured human colon carcinoma cells. *Cancer*. 1985;56:1559–1565.
33. Putignani L, Raffa S, Pescosolido R, et al. Preliminary evidences on mitochondrial injury and impaired oxidative metabolism in breast cancer. *Mitochondrion*. 2012;12:363–369.
34. Poli G, Guasti D, Rapizzi E, et al. Morphofunctional effects of mitotane on mitochondria in human adrenocortical cancer cells. *Endocr Relat Cancer*. 2013;20:537–550.
35. Shapovalov Y, Hoffman D, Zuch D, et al. Mitochondrial dysfunction in cancer cells due to aberrant mitochondrial replication. *J Biol Chem*. 2011;286 (25):22331–22338. doi:10.1074/jbc.M111.250092.
36. Dardick I, Caldwell D, Bailey D, et al. Nuclear morphologic and morphometric analyses of nodular poorly differentiated lymphocytic lymphoma: assessment of small cleaved nuclei. *Hum Pathol*. 1985;16:1187–1199.
37. Fischer A, Bardarov S, Jiang Z. Molecular aspects of diagnostic nucleolar and nuclear envelope changes in prostate cancer. *J Cell Biochem*. 2004;91:170–184.
38. Finol H, Marquez A, Bello B, et al. Ultrastructure of Skeletal Muscle Alterations Surrounding a Malignant Fibrous Histiocytoma. *J Exp Clin Cancer Res*. 1994; 13: 381–384
39. Dakubo G, Jakupciak J, Birch-Machin M, et al. Clinical implications and utility of field cancerization. *Cancer Cell Int*. 2007;7:244–256.
40. Martinez-Outschoorn U, Balliet R, Rivadeneira D, et al. Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution. *Cell Cycle*. 2010;9:3276–3296.