Artículo Regular

COLON ADENOCARCINOMA AND CELL DEATH TYPES: AN ULTRASTRUCTURAL STUDY

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Recibido: Septiembre 2015. Aprobado: Abril 2016.
Publicado: Junio 2016.

ABSTRACT
Three forms of cell death were found in human colon adenocarcinoma (HCAC), apoptosis, autophagy, and necrosis, by transmission electron microscopy. Apoptosis was characterized by chromatin hypercondensation and formation of apoptotic bodies, which were covered by plasma membrane. In autophagic cell death, autophagosomes of different complexities were observed along the process of cell degeneration which preceded nuclear collapse. In necrosis, cytoplasmic organelles, most notably mitochondria, were swollen, and plasma membrane was broken down causing the release of the cellular content into the extracellular space.

Keywords: Human colon adenocarcinoma**,** ultrastructure**,** apoptosis**,** autophagy**,** necrosis

ADENOCARCINOMA DE COLON Y TIPOS DE MUERTE CELULAR: UN ESTUDIO ULTRAESTRUCTURAL

RESUMEN
Tres formas de muerte celular se encontraron en adenocarcinomas de colon humano (HCAC), la apoptosis, la autofagia y la necrosis, mediante microscopía electrónica de transmisión. La apoptosis se caracterizó por la hipercondensación de la cromatina y la formación de los cuerpos apoptóticos, los cuales estaban cubiertos por la membrana plasmática. En la muerte por autofagia, autofagosomas de diferentes complejidades fueron observados a lo largo del proceso de degeneración celular que precedió al colapso nuclear. En la necrosis, los organelos citoplasmáticos y más notablemente las mitocondrias, se hincharon, mientras que la membrana plasmática se rompió y el contenido celular se liberó hacia el espacio extracelular.

Palabras clave: Adenocarcinoma de colon humano, ultraestructura, apoptosis, autofagia, necrosis

INTRODUCTION

Cell death has been classified as apoptotic, necrotic or autophagic according to its morphological appearance. Apoptosis was the first identified genetically programmed cell process being the principal mechanism by which cells are eliminated in physiological conditions in metazoan organisms [1,2]. Autophagy, a process known to provide survival capability to cells in nutrient deprivation, has also been recently linked to the death process [1]. In association with apoptosis, autophagy may act as a tumor suppressor [3]. On the contrary, autophagy also is essential for the survival of cancer cells, which show a very high level of autophagic process. Autophagosome formation is most prominent in tumors growing in hypoxic environment [4]. In this work we present ultrastructural results of the coexistence of apoptosis, necrosis and autophagy in tumors from patients with HCAC.

MATERIALS AND METHODS

Patients admitted to the study were attending the Coloproctology Unit of Caracas University Hospital. The Ethics Committee of the Hospital approved the project

and the subjects gave written consent. For the present work, colon biopsies were taken from four (4) male patients (ages between 45 and 75 years), with a diagnosis of HCAC in stages C and D according to Dukes' system modified by Gunderson and Sosin [5]. The samples were stretched between two pins, covered for 5 min with Karnovsky fixative in a 320 mOsmol Millonig phosphate buffer, pH 7.4. After that, the samples were diced into small blocks (2mm length X 1mm diameter), fixed in 1% Karnovsky fixative, postfixed in 1% OsO_4, dehidrated in ethanol and embedded in EMbed-812 resin (EMS, Hatfield, Pennsylvania). Sections were cut with a diamond knife in a Porter-Blum MT2-B ultramicrotome and stained with uranyl acetate and lead citrate. Sections were observed in a Jeol JEM-1011 transmission electron microscope, at an accelerating voltage of 80 kV.

RESULTS AND DISCUSSION

In our study of epithelial cells from HCAC we found, in addition to apoptosis as the classical form of programmed cell death I, autophagy and necrosis, by the use of transmission electron microscopy. Distinction of a dying cell as apoptotic has been considered indisputable if the cell meets ultrastructural criteria [6]. Nevertheless one of the disadvantages of this method is that only a small portion of the sample can be seen at one time. Resin –embedded tissue sectioned at 1-3 µm and stained with toluidine blue or methylene blue can be used in order to identify intensely stained apoptotic cells observed by light microscopy [6]. One disadvantage is that this method underestimates the extent of apoptosis because cell fragments or apoptotic bodies are not detected. In the case of labeling DNA by TUNEL method, several studies have shown that it can not always be possible to distinguish apoptotic and necrotic cells [7, 8].

In Fig. 1 – 4, it is possible to observe different stages (or degrees) of apoptotic process in colon tumor cells, including a nucleus with a hypercondensed chromatin and the formation of apoptotic bodies containing chromatin condensation and cytoplasmic fragments. Similar content of apoptotic bodies was found in human colorectal tumors [9] and in the provoked mode of cell death induced in murine intestinal crypts by cytotoxic drugs as cytosine arabinoside, vincristine, adriamycin and nitrogen mustard [10].

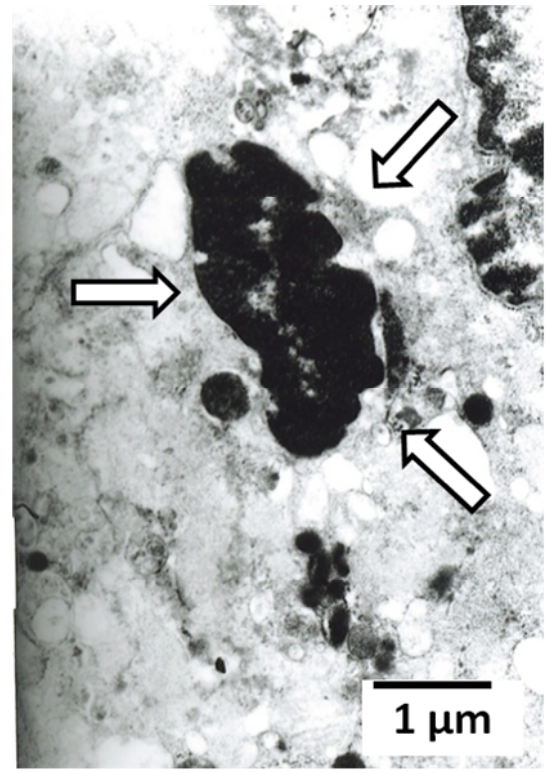

Fig. 1. Observe the hypercondensed nucleus of an apoptotic cell (arrows).

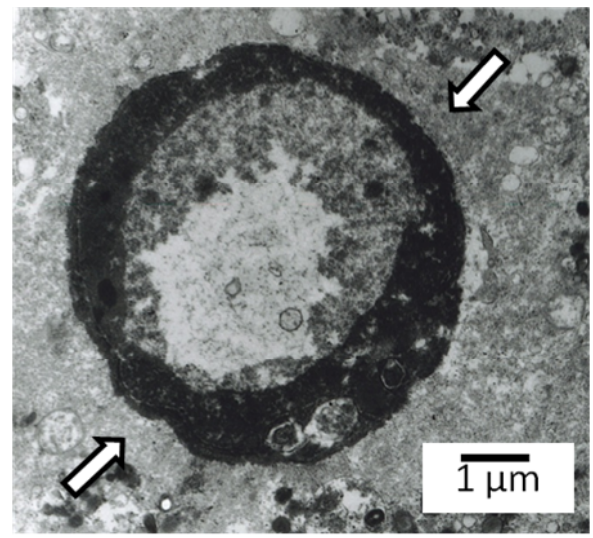

Fig. 2. Toroid structure formed by chromatin condensed around the periphery of the nucleus (arrow).

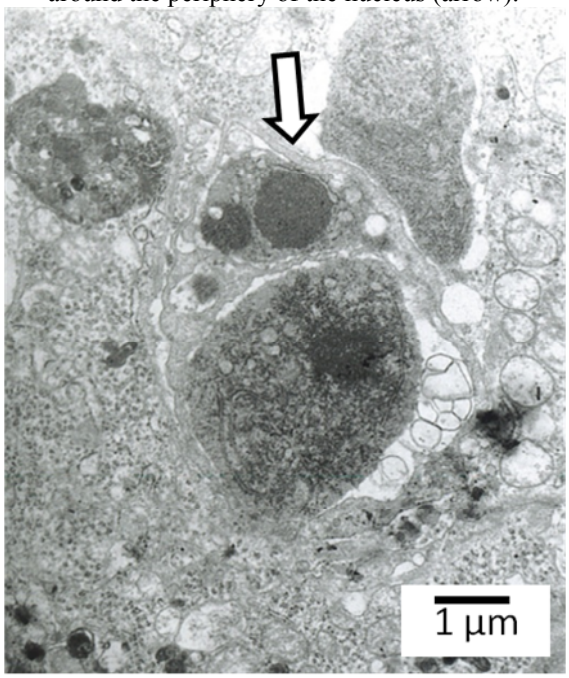

Fig. 3. Apoptotic body with condensed chromatin (arrow).

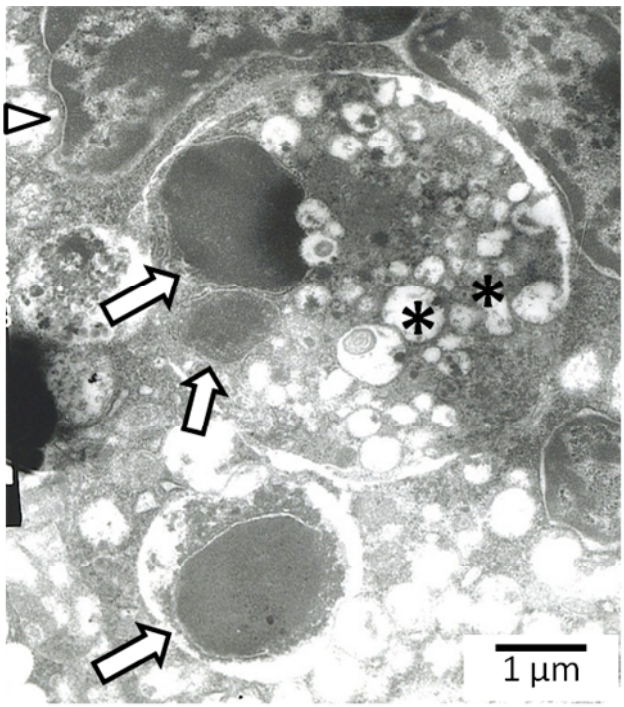

Fig. 4. Apoptotic bodies with chromatin fragments (arrows) and altered organelles (asterisks). They are located intracellularly after phagocytosis by a neighboring epithelial cell (arrowheads).

The comparison between proliferation and apoptosis in colorectal carcinoma has shown that apoptotic and proliferative indices are higher in metastatic foci than in the primary colorectal carcinomas [11] These results would suggest that apoptosis reflects not only cell death but also the proliferation of colorectal carcinomas. In autophagic dying cells, disappearance of organelles was seen with formation of autophagosomes of different complexities (Fig. 5 and 6). The autophagosomes are double membrane vacuoles formed in the cytosol that encapsulate whole organelles such as mitochondria and bulk cytoplasm. This type of autophagy is known as macroautophagy [12].

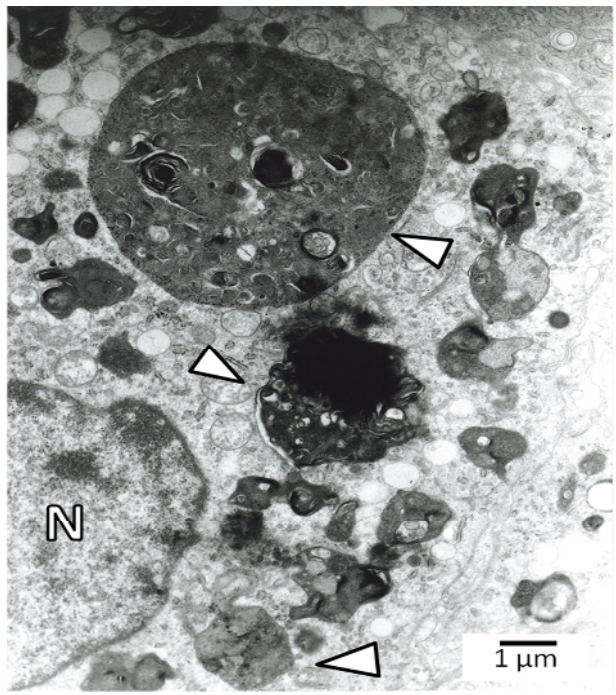

Fig. 5. Section of a cell in the process of autophagy. Autophagolysosomes (arrowheads) present different degrees of complexity. Note that the nucleus has not yet collapsed (N).

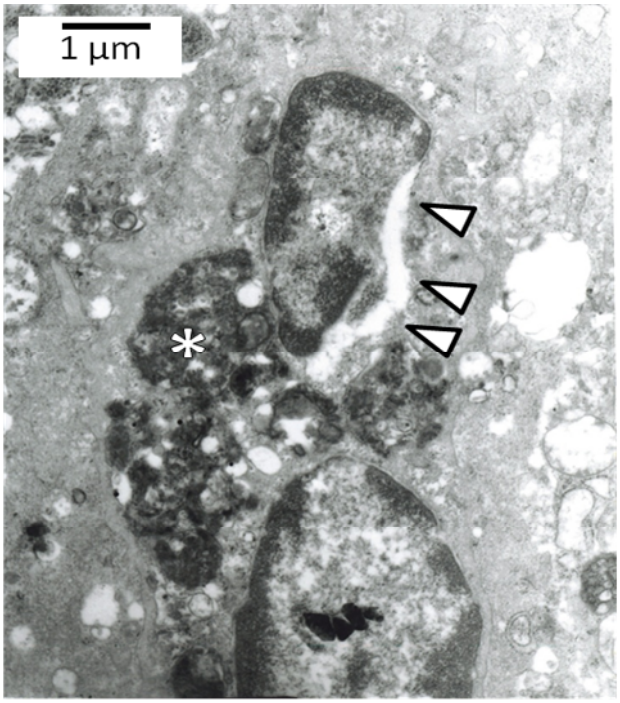

Fig. 6. Autophagolysosomes present a more digested content (asterisk). The nucleus shows swelling of envelope with breakdown of the external membrane (arrowheads).

Increased autophagy, the hallmark of programmed cell death type II led to cell death via destruction of cytoplasm. Early destruction of cytoplasm preceded nuclear collapse but it has been described that it may also occur without it [13]. As in the case of apoptosis, a cell undergoing autophagic cell death may not complete this type of process ending in a secondary necrosis [14]. In the context of cancer, autophagy appears to play an ambiguous role. In association with apoptosis, autophagy can act as a tumor suppressor. On the other hand, defects in autophagy, in concert with abnormal apoptosis, may trigger tumorigenesis and also therapeutic resistance [15,16]. Necrosis occurred with breakdown of plasma membrane, cell and organelle edema, and the release of cytoplasm content into the extracellular space. (fig. 7). This process looks like a passive and uncontrolled form of cell death and because of that necrosis has for long been considered as an accidental mode of cell death. Nevertheless, a growing body of scientific research has implicated lately a tight regulation of necrotic processes similar to apoptosis [17], therefore

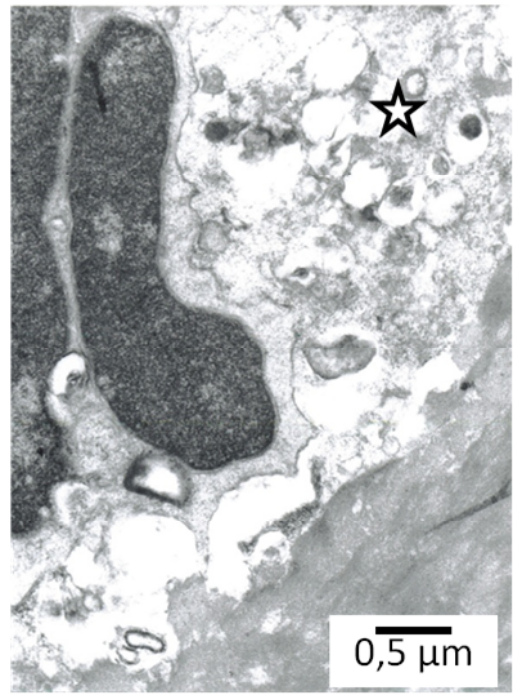

Fig. 7. See necrotic rests (star).

a programmed form of necrosis, termed necroptosis, has been recognized according to morphological criteria that can be regulated via signal transduction pathways [18]. Necroptosis has been suggested as an alternative form of programmed death even under conditions when apoptosis is blocked [17]. Inhibition of caspase activity, one of the hallmarks of apoptosis, enhances rather than inhibits necroptosis. A decade of cell death research has shown that necrosis, apoptosis and autophagy are regulated by similar pathways using the same proteins [4]. Defects in cell death signaling have been considered a fundamental phenomenon in the progress of colorectal cancer.

CONCLUSION

Our results indicate that three forms of cell death are present in HCAC, apoptosis, autophagy and necrosis. It was possible to distinguish the three forms of cell death on ultrastructural grounds alone. Our results support the view according to which transmission electron

microscopy remains the "gold standard" for identification of the specific features of cell undergoing death [19], and for categorization of a dying cell as apoptotic, autophagic or necrotic.

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