

# Ultrastructure of Liver Metastases from Colon Adenocarcinomas

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## ABSTRACT

Liver metastases from colon adenocarcinoma cells exhibited features of primary tumor site such as microvilli with filamentous cores and long rootlets, glycocalyceal bodies, and classical desmosomes. Nevertheless, some cell surfaces showed scarce and short microvilli. Attenuated and imperfect desmosomes were also seen as well as swelling of rough endoplasmic reticulum, Golgi apparatus cisternae and mitochondria with electron-dense granules and scarce cristae, and the proliferation of lysosomal myelin-like figures and multivesicular bodies. In some areas, neoplastic cell entered into contact with hepatocytes and macrophages. It is concluded that in liver the invasive phenotype produced metastatic foci with cellular distinctive aspects, several of them different from the primary tumor.

**Key words:** Colon adenocarcinoma, liver metastases, ultrastructure

## INTRODUCTION

The liver is the most common place of metastases from the gastrointestinal tract, being the most frequent diagnosis of adenocarcinoma.<sup>[1]</sup> Nevertheless, liver metastases from leiomyosarcoma are also frequent.<sup>[2-4]</sup> Cell forming metastases show differences in comparison with these belonging to the primary tumor.<sup>[5]</sup> This finding supports the existence of an invasive phenotype limited to more aggressive subpopulations of neoplastic cells.<sup>[6]</sup> This phenotype could have distinct morphological characteristics and, under some circumstances, metastases could be different enough from primary neoplasm as to cause difficulty in diagnosis or even misdiagnosis in pathological examinations by light microscopy.<sup>[7]</sup> The problem could be even more complicated if metastases themselves had the capacity to metastasize.<sup>[8]</sup> This difficulty has been presented in colorectal adenocarcinomas when they are compared with metastases from adenocarcinomas of the lung and other organs. For

this reason, features are needed to allow the differentiation of these tumor, and this conducted to the determination of the ultrastructural markers of colon adenocarcinomas which could help to distinguish colorectal adenocarcinomas from other carcinomas sole on ultrastructural grounds.<sup>[9]</sup> Hepatic metastasis from human colon adenocarcinoma implies a poor prognosis.<sup>[10]</sup> Nevertheless, in the pathological literature, we could not find a systematic ultrastructural study in relation to differences or similarities between human colon tumor and it's hepatic metastases in terms of cellular findings. Only some descriptions were reported using intact patient colorectal tissue or human colon carcinoma lines injected into nude mice.<sup>[10-12]</sup>

In the present work, it is described the hepatic metastases ultrastructure from human colon adenocarcinomas which showed variations from alterations already observed in primary tumors or some cases, not observed in them.

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## MATERIALS AND METHODS

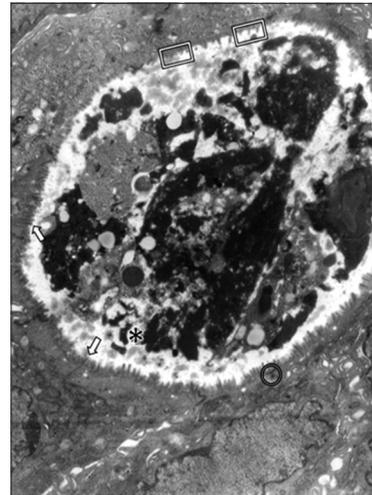
For the realization of this study, biopsies were taken from 10 ( $n = 10$ ) patients affected by colon adenocarcinomas and diagnosis of hepatic metastases. Biopsies obtained from liver metastases were processed with routine techniques for transmission electron microscopy, including fixation with 3% glutaraldehyde in a 320 m Osmol phosphate buffer, pH = 7.4 and postfixed in 1% OsO<sub>4</sub>, dehydrated in ethanol and embedded in LX-122 resin (LADD Res. Inc., Burlington). Sections were cut with a diamond knife in a Porter-Blum MT2-B ultramicrotome, stained with uranyl acetate and lead citrate and observed in a Hitachi H-500 transmission electron microscope at an accelerating voltage of 100 kV. Three grids were obtained from each block, and about 10 micrographs were made from each grid. Thick sections (3–5 μm) were stained with toluidine blue for light microscopy.

## RESULTS

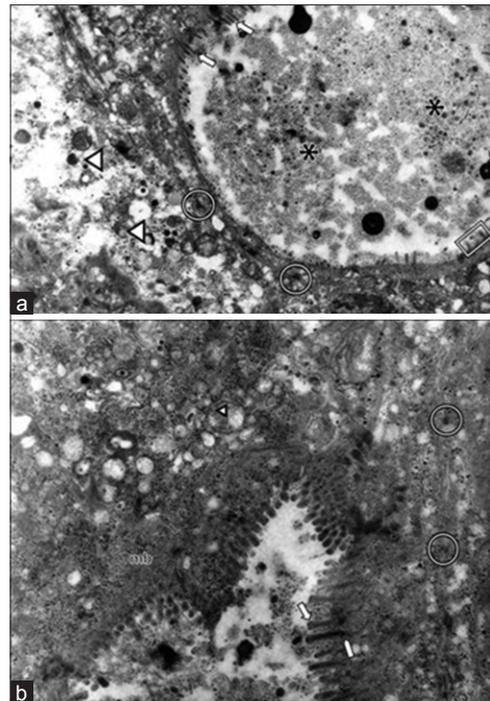
In liver, foci of neoplastic cells from colon adenocarcinomas exhibited the typical features from primary tumors as microvilli with microfilamentous cores and long rootlets, glycocalyceal bodies, and desmosomes [Figures 1-3]. Microvilli were in some lumens more prominent than in others [Figures 1,2]. Some of the cells showed an almost flat apical surface with scarce and short microvilli [Figure 1,2]. Desmosomes and attenuated and imperfect desmosomes<sup>[13,14]</sup> were seen [Figure 3,4]. Attenuated desmosomes usually show slender dense plaques. On the contrary, imperfect desmosomes exhibit very thick plaques. Cells also presented changes not previously described in primary tumors,<sup>[9]</sup> as swelling of rough endoplasmic reticulum (RER), Golgi apparatus and mitochondria [Figures 2-4], the presence of scarce mitochondrial cristae and electron-dense granules, and lysosomal proliferation, including myelin-like figures and multivesicular bodies [Figure 5]. In some places, neoplastic cells and hepatocytes contacted, the latter ones exhibiting swollen RER and mitochondria with a very electron-dense matrix free of cristae [Figure 6]. In areas next to the basement, membrane cells showed abundant RER and ribosomes and presented microvilli with interruption of basement membrane [Figure 6]. In this Figure 6, microvilli contact the macrophage surface.

## DISCUSSION

Short cell surface microvilli are not considered absolutely pathognomonic of intestinal adenocarcinoma,<sup>[15]</sup> because they were seen in pulmonary adenocarcinomas. However, long rootlets associated with other feature as abundant glycocalyceal bodies are been considered by Hickey and Seiler<sup>[9]</sup> as pathognomonic of large intestinal-type adenocarcinoma. In the hepatic metastases, short microvilli

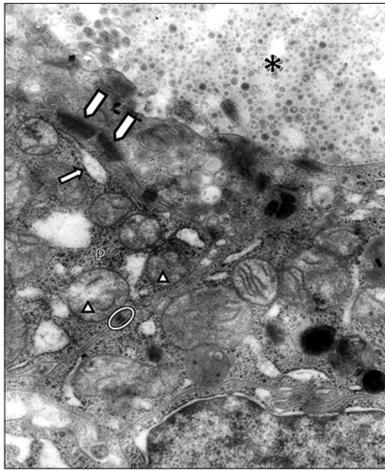


**Figure 1:** In this section: Short microvilli (Rectangles), desmosome (Circle), long rootlets (Arrows), note cell debris in the lumen (Asterisk), 12000X

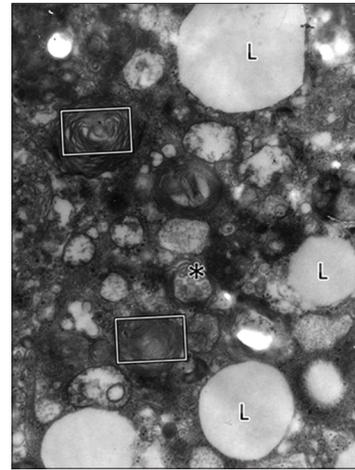


**Figure 2:** (a) In this section: Lumen with glycocalyceal bodies (Asterisks), area of cellular necrosis (Triangles), long microvilli (Arrows), short microvilli (Rectangle), and desmosomes (Circles). 14000X. (b) In this section: Microfilamentous core (Arrow) with long rootlet (Pentagonal Arrow), swollen mitochondria (Triangle), multivesicular body (mb), and desmosomes-like structures (Circles), 30000X

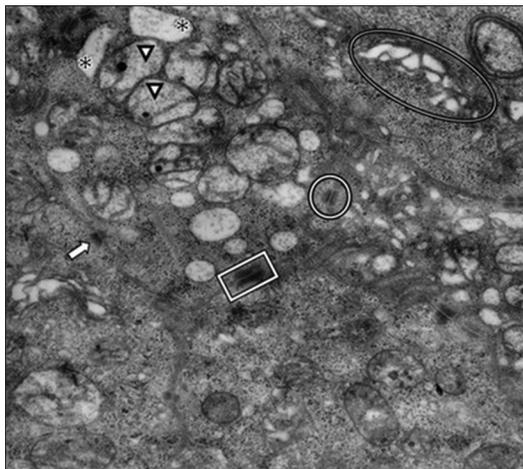
and microvilli with long rootlets were observed by us associated with numerous glycocalyceal bodies and due to this reason it should not be considered as pathognomonic of primary colon adenocarcinoma. Metastasis of human colon carcinoma cell line LM-H3 showed tumor cells



**Figure 3:** In this section: Glycocalyceal bodies (Asterisk), imperfect desmosome (Pentagonal arrows), attenuated desmosome (Oval), swollen mitochondria (Triangles), swollen rough endoplasmic reticulum (Arrow), and polysomes (P), 36000X

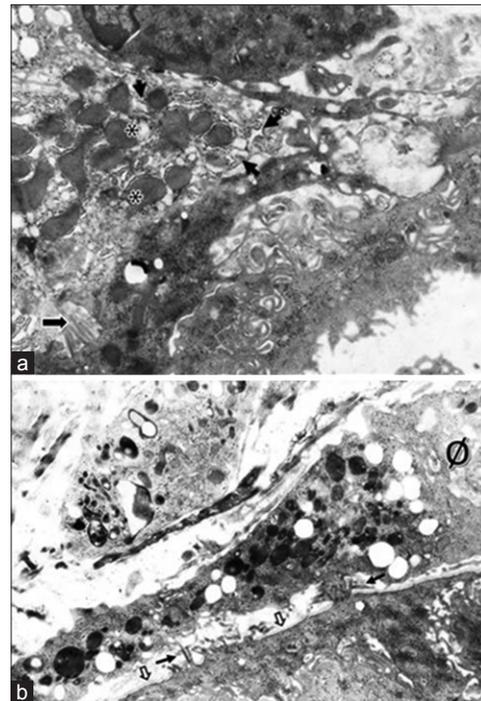


**Figure 5:** In the cytoplasmic region: Myelin-like figures (Rectangles), Multivesicular body (Asterisk), Note abundant lipid droplets (L), 36000X



**Figure 4:** In this section: Swollen mitochondria with electron-dense granules (Triangle), swollen rough endoplasmic reticulum (Asterisks), desmosome (Circle), imperfect desmosome (Rectangle), attenuated desmosome (Arrows), and swollen Golgi apparatus (Oval), 30000X

with microvilli and forming bile canaliculi with adjacent hepatocytes.<sup>[11]</sup> On the contrary, in our study nests with lumen were formed only by tumor cells, similarly to the observed in metastatic foci in the lung of athymic nude mice injected with T84 and T84SF human colon carcinoma cellsh.<sup>[16]</sup> In the work of Shimizu *et al.*,<sup>[11]</sup> classical desmosomes were described but no attenuated or imperfect ones as observed by Ghadially.<sup>[13]</sup> The number of desmosomes can be increased in cultured colon cancer cells by administration of dimethylformamide.<sup>[17]</sup> This ultrastructural evidence suggests that this junction is not a static feature but could be adapted to different situations and that it is related to the behavior of tumor cells. In liver metastasis of nude



**Figure 6:** (a) In this section: Neoplasm cell with long microvilli (Arrow), Mitochondria from a hepatocyte (Asterisk), Swollen rough endoplasmic reticulum cisternae (Arrowheads) 12000X. (b) Basement membrane (White arrows), Microvilli on neoplastic cell (Black arrows), and Macrophage (Ø), 10000X

mice model from human rectal carcinoma (HRA-HMN-1), Qiu-Zhen *et al.*<sup>[12]</sup> found cancer exhibiting desmosome-like intracellular junctions as these described by Ghadially as desmosome-like structures.<sup>[13]</sup> Contrary to the finding of Ghadially *et al.*,<sup>[14]</sup> we did not find imperfect desmosomes forming part of giant desmosomes.

In opposition to our findings, in liver metastasis models of human colorectal carcinomas, only moderate amount of RER was observed in tumor cell cytoplasm.<sup>[12]</sup> The study of T84 human colon carcinoma cells showed that cytoplasm contained some RER elements and free scattered ribosomes.<sup>[16]</sup> On the contrary, abundant RER elements, free ribosomes, and polysomes were located by us in the throughout the cytoplasm. Furthermore, present was swollen cisternae of Golgi apparatus and numerous swollen mitochondria which contained electron-dense granules in the matrix as it was described in swollen mitochondria of hepatic metastases from a colon leiomyosarcoma.<sup>[3]</sup> Changes in hepatocytes contacting tumor cells were similar to those described in perimetastatic areas in relation to swelling of RER and mitochondria devoid of cristae,<sup>[18]</sup> supporting the point of view that the non-invaded cells in close proximity to metastases were also abnormal.

Cancer-related inflammation is characterized by the recruitment of macrophages to the primary tumor and to premetastatic niche, to favor secondary location of cancer.<sup>[19]</sup> The presence of tumor-associated macrophages and contacting tumor cells as was seen in this work could indicate promotion of metastasis.<sup>[20]</sup> Cells which metastatize are limited to the most aggressive type and form the so-called invasive phenotype.<sup>[6]</sup> Therefore, we can conclude that in liver the invasive phenotype produced metastatic foci with cellular distinctive aspects and several features different from those found in primary tumor cells.

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