COMPARISON OF ELECTRON MICROSCOPIC OBSERVATION BETWEEN EXPANSIVE TYPE AND INFILTRATIVE TYPE OF HEPATOCELLULAR CARCINOMA

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ABSTRACT

Objective: To study the ultrastructure and biological characteristics of expansive type of hepatocellular carcinoma (EHCC). Methods: Examination of EHCC and infiltrative type of hepatocellular carcinoma (IHCC) (each 20 cases) by electron microscope (EM) to compare their ultrastructure. Results: The 40 cases were divided into 3 groups: 16 cases of well differentiated EHCC, 4 cases of poorly differentiated EHCC, and 20 cases of poorly differentiated IHCC. The ultrastructure of well differentiated EHCC was similar to the surrounding non-cancer hepatocytes; the characteristics of them were as follows: 1. Cell membrane was developed well and cell border was clear; 2. Round nucleus was of regular shape; nuclear membrane was smooth; 3. Nucleoli were round, regular and bigger than normal; and 4. Plentiful endoplasmic reticulum and mitochondria were well developed. The ultrastructure of poorly differentiated EHCC and IHCC were identical: 1. Membrane was poorly developed; 2. Irregular nuclei were deeply indented or lobulated and many pseudoinclusions were seen; 3. Majority of the nucleoli were big, sponges or ring-formed; 4. Organelles were plentiful or scanty and tended to be degenerated. Conclusion: Most of the EHCC were mature by EM observation; this explained the EHCC's slow growth pattern, but some still had invasive potential.

Key words: Hepatocellular carcinoma, Expansive type, Ultrastructure

Expansive hepatocellular carcinoma (EHCC) is a type of HCC with favorable prognosis. Since it was first reported at the end of 1970s',[1-3] many scientists have paid attention to its biological characteristics. The ultrastructure of HCC were earlier reported in the 1960s' including case reports,[4] systematic cases were analyzed[5-9] and special case report etc. But as far as we know, there still has been no systematic report of the ultrastructure of EHCC in the Literature. We studied 20 cases of EHCC and 20 cases of IHCC by transmission electron microscope to find the relationship between ultrastructure of EHCC and their biological characteristics.

MATERIALS AND METHODS

Materials

Twenty cases of EHCC and 20 cases of IHCC were obtained in 1990-1998 from the Pathology Department of Shantou University Medical College. All cases of EHCC met the Kojiro's criteria of HCC in expansive type.[9] Grossly cancer nodules of all cases were distinctive from the surrounding tissue and encapsulated by fibrous tissue; on the other hand, all of the IHCC cases had no border between the cancer tissue and liver tissue. 18 cases of EHCC had single nodules; 2 cases had multiple nodules: the size of the cancer nodule was from 0.5 cm to 20 cm. Under light microscopic observation, all cases of EHCC had fibrous capsules; 15 cases of them had micro-cancer, two of 15 cases had broken through the capsule and infiltrated the non-cancerous hepatic tissue. According to the improved Edmondson criteria,[10] in the 20 cases of EHCC, grade I there were 2 cases, grade II there were 14 cases, grade III there were 4 cases; while in the 20 cases of IHCC, 17 cases were grade III, and 3 cases were grade IV. Evaluate the differentiation of EHCC and IHCC more effectively, Jiang Zhenghui's criteria were also used;[11] grade I and grade II are well-differentiated type, grade III and grade IV are poorly-differentiated type. Of the 20 EHCC cases, 16 cases belonged to the well-differentiated type, 4 cases with poorly- differentiated type; all 20 cases of IHCC belonged to the poorly-differentiated type.

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Methods

According to the routine electron microscope technique, we cut a block of cancer tissue and surrounding hepatic tissue respectively from each case. The blocks were double 2.5% with glutar aldehyde and 1% osmic acid double fixed, dehydred and embedded, sectioned and stained. The specimens were studied with a by H-300 transmission electronic microscope.

RESULTS

Surrounding Hepatic Tissue EM Observation

The ultrastructure of all surrounding tissues of EHCC and IHCC were similar. Hepatocytes were polyhedral; the cell membrane was well developed; there were tight junctions and desmosomes between neighboring cells; many microvilli were located on the face of bile canaliculi; organelles were plentiful, mitochondria and endoplasmic reticula were well developed; lysosomes, glycogen and fat granules were seen in the plasma; nuclei were regular, single, round or oval; double nuclei occurred occasionally; nuclear membrane was smooth, no infold, prominence or groove, both euchromatin and heterochromatin were seen; nucleoli were regular, round and solid.

EHCC EM Observation

Under light microscope (LM), there were 16 cases of well-differentiated type (grade I, II) and 4 cases of poorly-differentiated (grade III) type among the 20 cases of EHCC. Under the EM, cancer cells of grade I and grade II had similar structure and both of them were similar from the surrounding hepatic tissue. The structures of poorly differentiated type were distinctively different to the surrounding hepatic tissue. The well differentiated type had following features: (1) Cell membranes were well-developed; neighbor cell's membrane were tightly juxtaposed; the majority of cells had distinctive border; desmosomes and other cell junctions were seen occasionally (Figure 1 and 2); many microvilli were seen in the canaliculi (Figure 3); (2) Nucleus was regular and round, the majority of cancer cells were of single-nucleus; the nuclear membrane was smooth, no indentation or prominence, similar to the surrounding hepatoyte. Nuclear chromatin was euchromotin (Figure 2); (3) Nucleoli were hypertrophic, but were still regular, round and solid. Fewer cases had abnormal nucleoli, such as reticular, spongy or loop-shaped nucleoli (Figure 4); and (4) Organelles were plentiful, rough endoplasm reticular were many more than normal; sometimes they had many local laminated or concentric arrangements (Figure 5). A lot of cases had more well developed mitochondria, but occasionally they were scanty and degenerated. No lysosome and few glycogen granules were seen in the plasma, but fat granules might occasionally occur.

The ultrastructures of poorly differentiated EHCC were similar to that of poorly differentiated IHCC.

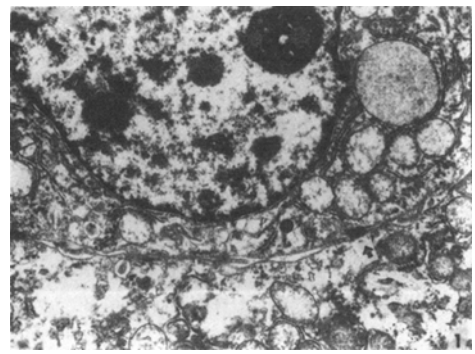

Fig. 1. Well-differentiated EHCC, well developed with cell membrane. Two desmosome between cells (↑) × 10000

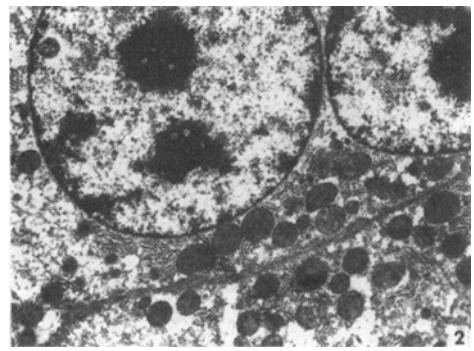

Fig. 2. Well-differentiated EHCC with double-nuclei, smooth nuclear membrane, well-developed organelle × 7000

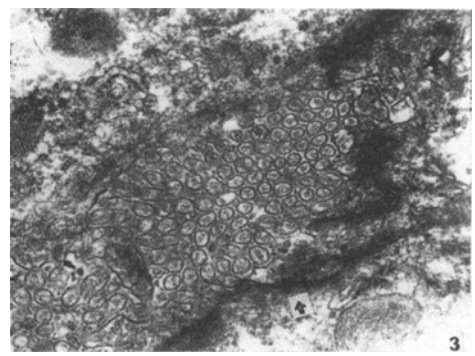

Fig.3. Well-differentiated EHCC with well-developed microvilli in the bile canaliculi, and tight junction around (↑) × 7000

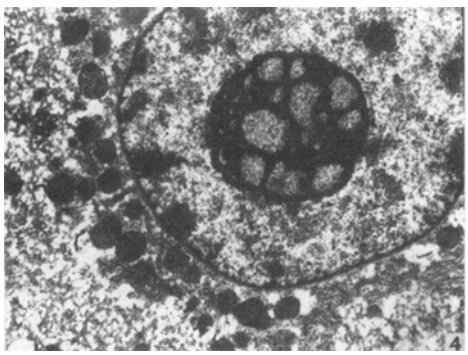

Fig 4. Well-differentiated EHCC with regular round nucleus, thinner reticulate nucleoli × 7000

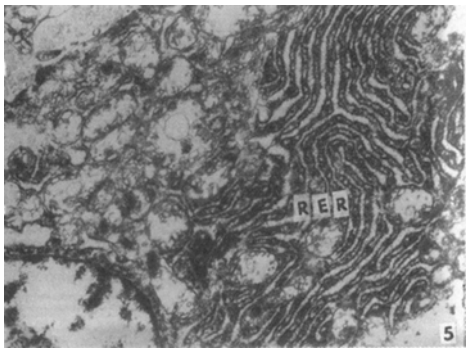

Fig. 5. Well-differentiated EHCC with plentiful and formly laminate rough endoplasm reticula × 10000

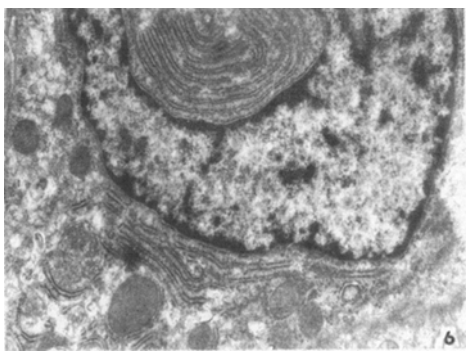

Fig. 6. Poorly-differentiated IHCC with abnormal indented nucleus. ×15000

IHCC EM Observation

All twenty IHCC cases belonged to poorly-differentiated by LM. The ultrastructure was very distinctively different from the surrounding hepatic tissue and well-differentiated EHCC: (1) Cell membrane was poorly developed, no distinctive border between cells, no desmosome or any other cell junction and no microvilli seen either; (2) Nuclei was irregular; nuclear membrane was bent, indent, prominent or rough. The contour looked jagged lobulated or "Buddha hand" shaped and pseudoinclusions of nucleus were also seen. Atypia of nuclei was the most distinctive change of the IHCC (Figure 6 and 7); (3) Majority of the nucleoli were irregular, huge, reticular, spongy or ring-form shaped; and (4) Organelles such as mitochondria and endoplasm reticula had more or less, but most of them were degenerated, scanty or poorly developed. Mitochondria lacked crista with scanty matrix (Figure 8); Endoplasm reticula were dilated and degranulated. No lysosome and glycogen granules; few fat granules were seen.

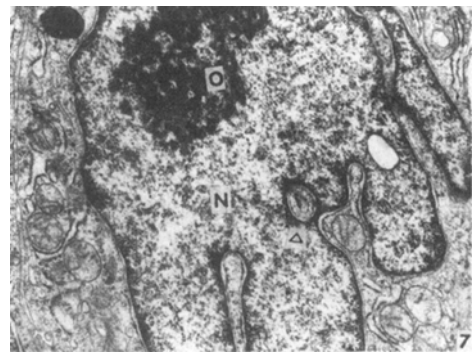

Fig. 7. Poorly-differentiated IHCC, nucleus is Buddha's hand abnormality (N), pseudoinclusion be formed (△), nucleoli rough reticule abnormality (O) ×15000

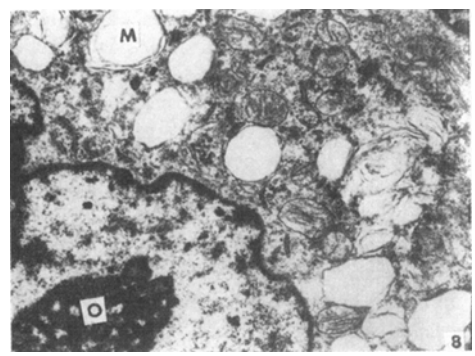

Fig. 8. Poorly-differentiated IHCC, mitochondria is plentiful, but majority were scanty and degeneration (M). nucleoli (O) was sponges abnormality ×15000

DISCUSSION

Growth pattern and differentiation are the two important indicators of tumor malignancy. These two indicators have a consistent relationship usually, but there are different ideas about the differentiation of

EHCC tissue. Okuda, et al.[1] thought that EHCC was well differentiated, but Ng, et al[2] pointed out that it has no statistical meaning if it was compared with IHCC. In this article, under LM, 20 EHCC cases included 80% well-differentiated cases and just 20% poorly differentiated cases, and 20 IHCC cases were all of poorly differentiated type. To compare them statistically, we agree with Okuda's point that the differentiation of EHCC is better than that of IHCC.

In this article, the differentiation of cancer cell of both EHCC and IHCC by EM is consistent with LM. According to the ultrastructure of cancer cells, 40 cases of HCC can be divided into 3 groups: 16 cases of well-differentiated EHCC, 4 cases of poorly differentiated EHCC, 20 cases of poorly differentiated IHCC. Under LM, well-differentiated EHCC belongs to grade I and II; Their ultrastructure are very similar and both of them are also similar to the surrounding hepatic tissue. But there are also some differences, such as poorly developed cell membrane, few desmosomes and cell junction are seen; lysosomes are lacking, and some abnormal or hypertrophy nucleoli appear. These indicate that EHCC is really well differentiated but alternatively has worse differentiation to a certain extent. Ghadially, et al. also reported one case of well differentiated HCC with complete capsule similar to our cases. They pointed out that this tumor tended to decline and to lose some cell functions.[4] Under LM, poorly-differentiated EHCC and IHCC are all in grade III and IV. Their cell ultrastructures are similar, and very different from the surrounding tissues, indicating that their differentiation is not well. They have great differences with well-differentiated EHCC such as membrane development, nucleus and nucleolus regularity, and the amount and shape of organelles etc.

The cell membrane development of cancer cells in well-differentiated EHCC was well and, cell membrane juxtaposes tightly giving distinctive borders. Minority of the cancer cells has desmosomes and other cell-junctions; microvilli are seen in the bile canaliculi. In poorly-differentiated EHCC and IHCC, development of membrane is not good, no distinctive border between cells, no desmosomes, no other cell junctions and no microvilli. The development of cell membrane might reflect not only the exchanging function between intracellular and extracellular substances, but also reflect the maturation degree of cell differentiation. In well-differentiated EHCC, the ultrastructure is similar to the surrounding tissue, while in poorly-differentiated EHCC and IHCC, the membrane is immature, reflecting its malignancy.

Regularity of the cancer cells nucleus, can indicate the differentiation and malignancy.[5-7] In the well-differentiated EHCC, the nucleus is regular, round shaped; the nuclear membrane is smooth. In the poorly-differentiated EHCC and IHCC, the nucleus is very irregular, the membrane was bent, indented and prominent. Thus abnormal nuclear shapes such as jagged, lobulared and nuclear pseudo in clusion are evident. Bent and indented membrane indicate that the nucleus and plasma need more area for substance exchange; it is a common character of malignant cancer cell, and also is a major difference between poorly differentiated EHCC or IHCC and well-differentiated EHCC.

The nucleoli of well-developed EHCC are similar to that of the surrounding hepatic cells, which are generally regular, round and solid, but the former are more hypertrophic than normal. A few abnormal nucleoli are seen; these features indicate that the metabolism of this type of cells is very active. In the poorly differentiated EHCC and IHCC, the majority of nucleoli are huge, reticular and spongy or ring-formed, indicating the protein metabolism is very active, and undergoing some disturbance.

Amount and structure of organelles can indicate the differentiation and metabolism function of tumor cells. O'Conor, et al. found that the amount of tumor cell's organelles is positively related with the differentiation degree; a lot of organelle can be seen in the highly-differentiated HCC, and only a few organelles can be seen in the moderate-differentiated HCC and few organelle in the undifferentiated HCC.[8] But Ordonez, et al. found that the majority of HCC cases had plentiful organelles except undifferentiated tumors.[6] We have found that the amount of organelles has the tendency to decrease when the differentiation degree declines. There are plentiful organelles in the well-differentiated EHCC, but the amount of organelles in poorly-differentiated EHCC and IHCC are not regular, some have more and some less. However, the structure of organelles are often swollen, degenerated or poorly developed. It seems that changes of organelle structures might better reflect the poor differentiation of cancer cell.

Generally speaking, LM observation combining EM observation could identify 80% EHCC cases to be of the well-differentiated type, 20% EHCC cases to be of the poorly differentiated type. Cancer being well-differentiated perhaps explains why EHCC grows slowly and expansively, but this type of EHCC may have the potential of aggression. We also observed 4 cases of poorly differentiated EHCC, two of which had broken through the capsule and had infiltrated the surrounding hepatic tissues.

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