PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroentero, 2000; 6(4):569-571
World Journal of Gastroenterology
 ISSN 1007-9327

Comparative ultrastructural study of endoplasmic reticulum in colorectal carcinoma cell lines with different degrees of differentiation

Shu Feng[1] and Jin Dan Song[2]

Subject headings colorectal neoplasms; endoplasmic reticulum; ultrastructure; microscopy, electron; microscopy, electron, scanning; cell diff erentiation

Feng S, Song JD.Comparative ultrastructural study of endoplasmic reticulum in colorectal carcinoma cell lines with different degrees of differentiation. ***World J Gastroentero***, 2000;6(4):569-571

INTRODUCTION

The endoplasmic reticulum (ER) consists of a complex system of tubules, lamellae , and flattened vesicles, and has a variety of morphologies in different cells. It is believed to play a central role in the biosynthesis of cholesterol, phosph olipids, steroids, prostaglandins, membrane and secretory proteins[1]. C ancer cells have different functions and ultrastructure from their original cel ls[2-4]. The studies on ER membrane system of cancer cells are of great significance in understanding their malignant behavior. In the present work, the ultrastructural characteristics of ER in human colorectal carcinoma cell lines with different differentiation degrees were investigated.

MATERIALS AND METHODS

Materials

Well differentiated human colorectal carcinoma cell line CCL229 and poorly d ifferentiated cell line CCL227 were generous gifts from Dana-Farber Cancer Inst itute of Harvard Medical School, USA.

Methods

Cell culture The human colorectal carcinoma cell lines CCL229 and CCL227 were grown in Dulbecco's modified Eagle's medium (DMEM, G1BCO, BRL) suppleme nted with 10% calf serum and 2 kU/L gentamycin. Cell cultures were incubated a t 37℃ in a humidified atmosphere of 95% air and 5% carbon dioxide.

Transmission electron microscopy Exponential cells cultured in flasks were collected, washed thrice in phosphate buffer saline (PBS), and then fixed in 2.5% glutaraldehyde in a buffer containing 0.1 mol/L sucrose and 0.1 mol/L sodium cacodylate (cacodylate buffer, pH7. 4) for 2 h. After being washed in cacod ycate buffer, the cells were post-fixed in 1% osmium tetroxide for 30 min, dehydrated through graded alcohol and acetone, and embedded in Epon 812. Ultrathin sections were cut with glass knives on LKB 2088 ultratome, stained with uranyl ac etate and lead citrate and examined under a Hitachi H600 transmission electr on microscope.

Scanning electron microscopy Cells grown on coverslips were rinsed thrice in a buffer containing 60 mmol/L sodium citric acid, 25 mmol/L KCl, and 35 mmo l /L $MgCl_2$ (sodium citric acid buffer, pH 7.4), then fixed in 125 mmol/L potassi u m permanganate in sodium citric acid buffer for 7 min. Cells were rinsed in sodium citric acid buffer, dehydrated through graded alcohol, replaced with iso-amy l acetate, dried at CO_2 critical point in a Hitachi HCB-2 critical point drie r, gilded on a BIKO TB-3 ion film-plating machine and then examined under a Hi tachi S-450 scanning electron microscope.

RESULTS

Well differentiated colorectal carcinoma CCL229 cells appeared round or polygona l with many microvilli and pseudopodia and a large elliptic nucleus containing 1 -2 dense nucleolus. The cytoplasm contained some mitochondria, Golgi apparatus, lysosomes, polyribosomes as well as abundant ER. The ER consisted of a complex system of tubules, lamellae, and flattened vesicles distri buted throughout the cytosol. There was a great amount of vesicle-like and flattened cisternal ER in pseudopodia (Figures 1, 2).

More apparent heteromorphism was observed in poorly differentiated colorectal ca rcinoma CCL227 cell. There were less microvilli and pseudopodia on the cell surf ace. The ratio of nuclei to plasma was higher than that found in CCL229 cell. An abundance of free polyribosomes and less

[1]Microbial Engineering Department, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, Liaoning Province, China
[2]Key Laboratory of Cell Biology, Ministry of Public Health of China, China Medical University, Shenyang 110001, Liaoning Province, China
Dr. Shu Feng, female, born on 1965-02-09, graduated from China Medical University as a postgraduate and got a doctorate degree in 1996, and is now working as a professor at the Institute of Applied Ecology, Chinese Academy of Sciences, and has having 32 papers published
Supported by the National Natural Science Foundation of China, № 3904005, and "Hundred-Person plan" of Chinese Academy of Sciences, (№ 10989902)
Correspondence to: Dr. Shu Feng, Microbiol Engineering Department, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, Liaoning Province, China
Tel. +0086-24-23899941
Received 2000-03-08 **Accepted** 2000-04-27

of mitochondria, Golgi apparatus and l ysosomes were found in the cytoplasm, and its ER, which mainly consisted of vesi cles and short tubules was much less than that in well differentiated CCL229 cell (Figures 3, 4).

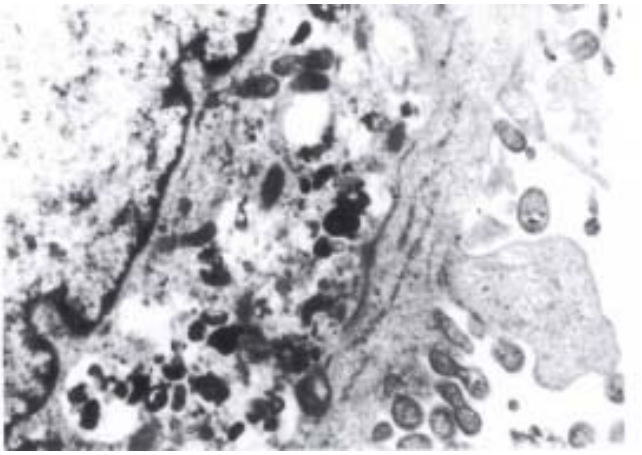

Figure 1 Ultrastructure of well differentiated colorectal carcinoma cell line CCL229 under TEM. × 800

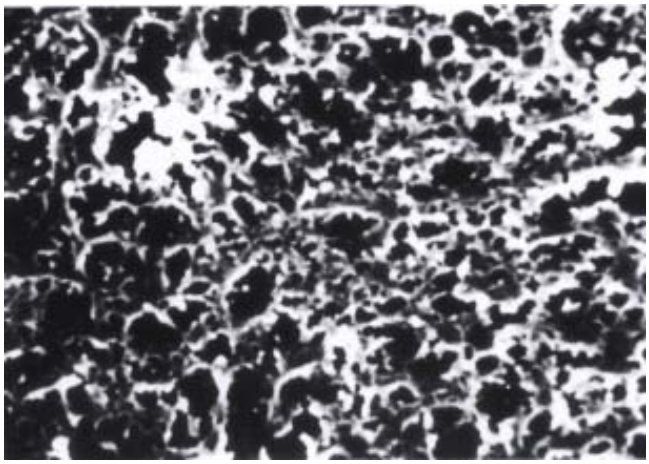

Figure 2 Ultrastructure of well differentiated colorectal carcinoma cell line C CL229 under SEM. × 7500

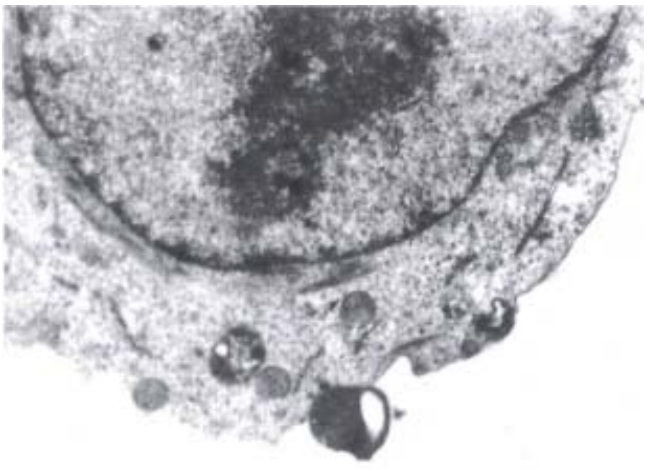

Figure 3 Ultrastructure of poorly differentiated colorectal carcinoma cell line CCL227 under TEM. × 500

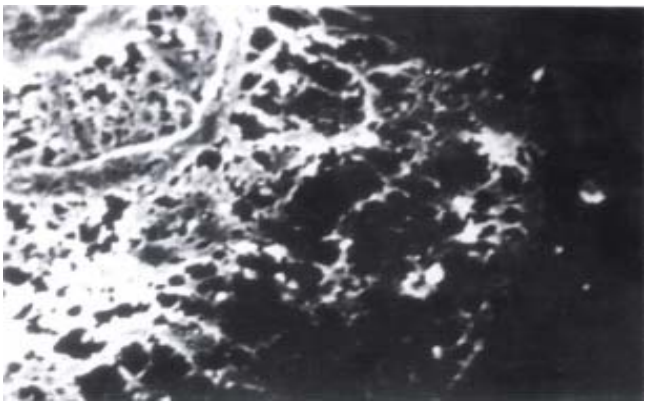

Figure 4 Ultrastructure of poorly differentiated colorectal carcinoma cell line CCL227 under SEM. × 5500

DISCUSSION

Overlap with other compartments and limits of regular light and electron microsc opy make it difficult to study the ultrastructure and cellular distribution of ER membrane system. Terasaki *et al*[5] reported a rapid and simple technique for localizing the structure of whole-mount ER both in living and gluta raldyde-fixed cells by fluorescent microscopy which can also be detected by pha se-contrast microscopy and scanning electron microscopy in potassium permangana te-fixed cells. With this technique, the ultrastructure and distribution of ER in many normal and tumor cells have been studied[6-11].

The ER is a highly specialized structure which performs many distinct functions. Hence a well developed ER may be looked upon as an expression of cell different iation and functional activity. It is abundantly clear that immature or undiffer entiated cells such as stem cells, embryonic cells, and cells in culture have, as a rule, a poor complement of ER as compared with their normal mature function ing counterparts, and this concept also applies to tumor cells. In general, there is a meaningful correlation between ultrastructural signs of anaplasia and mal ignancy. In this study, using regular transmission microscopy and whole-mount E R scanning electron microscopy of potassium permanganate fixation, the ultrastru cture and distribution of ER in well and poorly differentiated colorectal carcin oma cell lines CCL229 and CCL227 was investigated comparatively. The results sho wed that well differentiated cell line CCL229 had abundant endoplasmic reticulum , which consisted of a complex system of tubules, lamellae, and flattened vesicl es distributed throughout the cytoplasm. A great amount of vesicle-like and fla ttened cisternal endoplasmic reticulum were found in pseudopodia. Poorly differe ntiated cell line CCL227 had relatively less ER. This result is coincident with the above-mentioned concept.

In poorly differentiated CCL227 cell, the ER is not abundant, but free polyribos omes are very rich in the cytoplasm. This presumably reflects the active synthes is of endogenous proteins needed for cell growth and division.

Well differentiat ed cell CCL229 is a highly invasive colorectal carcinoma cell line, its high inv asiveness correlates to its cell's ultrastructure[12,13]. Pseudopodia of tumor cells play an important role in the invasion process[14], tumor cells are supposed to secrete a great amount of proteolytic enzymes to cleave the basement membrane[15-18]. In this work, cell CCL229 was found to have many microvilli and pseudopodia with abundant ER which presumably reflect its a ctive synthesis of secretory proteins. This may be the ultrastructural basis of its high invasiveness.

REFERENCES

1 Song JD, Chen YH, Sun BD, Chen C, Feng S, Ge CH, Wang YQ. Stu dies of some proteins synthesized by endoplasmic reticulum in cancer cells. *Zhongguo Yixue Shengwuxue Yanjiu,* 1998;191-193
2 Zhu YQ, Song JD. Microscopic and submicroscopic structure changes of endoplasmic reticulum in whole mount effected by carcinogen. *Zhonghua Wuli Y ixue Zazhi,* 1993;15:22-25
3 Li D, Song JD. Ultrastructure changes of endoplasmic reticulum in oncogene transfected cells. *Zhonghua Wuli Yixue Zazhi,* 1996;18:1-4
4 Zhu YQ, Song JD. Correlation between distribution of endoplasmic reticulum and cell phentotype *in vitro* neoplastic transformation. *Zhonghua Wuli Yixue Zazhi,* 1996;18:163-165
5 Terasaki M, Song JD, Wang JR, Weiss MJ, Chen LB. Localization of endoplasmic reticulum in living and glutaraldyde fixed cells with fluorescent dyes. *Cell,* 1984;38:101-108
6 Song JD, Lee C, Lin CHS, Chen LB. Electron microscopic studies of the endoplasmic reticulum in whole mount cultured cells fixed with potassium permanganate. *J Struct Biol,* 1991;107-109
7 Song JD, Chen LB. The structure of endoplasmic reticulum in whol emount cultured cells with potassium permanganate fixation. *Zhongguo Yike Da xue Xuebao,* 1986;1:1-4
8 Huang JQ, Song JD. Pleomorphism of tridimensional architecture of w holemount endoplasmic reticulum in cultured cells. *Jiepou Xuebao,* 1996;27:269-272
9 Song JD. The fine structure of endoplasmic reticulum in cells. *Zhongguo Yixue Shengwuxue Yanjiu,* 1995;94-95
10 Zhao YH, Song JD. Transmission electron microscopic studies of the whole mount endoplasmic reticulum in the cell cycle. *Jiepou KexueJinzhan,* 1998;4:80-84
11 Zhao YH, Song JD. Scanning electron microscopic studies on the endoplasm icreticulum in the cell cycle. *Zhonghua Wuli Yixue Zazhi,* 1995;17:165-167
12 Li YC, Wang YQ, Song JD. Morphological observation of endoplasmic reticu lum in human colorectal cancer cells with different invasive ability. *Jiepou K exue Jinzhan,* 1998;4:350-354
13 Sun BD, Song JD. Inhibition of invasiveness and expression of epidermal growth factor receptor in human colorectal carcinoma cells induced by retinoic a cid. *Cell Res,* 1995;5:135-142
14 Sun BD, Song JD. Ultrasructure of endoplasmic reticulum in human colorectal carcinoma cells with different invasiveness. *Zhonghua Wuli Yixue Zazhi,* 1997;19:43-44
15 Feng S, Wang YY, Song JD. Relationship between expression of laminin and pathological feature in human colorectal carcinoma. *World J Gastroentero,* 1998;4:219-221
16 Shu Feng, Jindan Song. Determination of β-glucuronidase i n human colorectal carcinoma cell lines. *China Nat J New Gastroentero,* 1997;4:251-252
17 Deng YJ, Li ZG, Qiu HM, Huang ZY, Ding YQ, Zhu MG. Comparison of meta static potentials of human colorectal carcinoma cell lines and their features re lated to metastasis. *Shijie Huaren Xiaohua Zazhi,* 1998;6:33-35
18 Feng S, Song JD. Relationship of β-glucuronidase to differentiation and invasion of human colorectal carcinoma. *Chin Med J,* 1999;112:854-857

Edited by Zhu QR
proofread by Mittra S