World Journal of Gastroenterology

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DOI: 10.3748/wjg.v6.iSuppl3.101

World J Gastroenterol 2000 September 15; 6(Suppl3): 101
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Components and distributions of cytoskeleton network in neoplastic Hep G_2 cells extracted with triton X-100 and $(NH_4)_2SO_4$

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Author contributions: Hai-Wei Zhang contributed solely to the work.

Supported by The National Science Foundation of China, No. 39500056.

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Received: December 24, 1999
Revised: March 10, 2000
Accepted: July 10, 2000
Published online: September 15, 2000

Abstract

AIM: To explore the components and the distributions of the cytoskeleton network in neoplastic Hep G_2 cells extracted with triton X-100 and $(NH_4)_2SO_4$.

METHODS: Using the mouse lung adenocarcinoma cell sublines (C_6/C_7) with low and high metastasis as a control, the human hepatocellular carcinoma cell line (Hep G_2) as well as the cell sublines (C_6/C_7) was extracted with triton X-100 and/or $(NH_4)_2SO_4$, then stained with Coomasie blue R250 or labeled with immunoenzymatic technique to identify the cytokeratin-type or vimentin-type intermediate filament components and study the distributions of cytoskeleton comparatively.

RESULTS: Extracted with triton X-100 and/or $(NH_4)_2SO_4$, then stained with Coomasie blue R250, the cells' cytoskeleton network were showed clearly; still it was very difficult to identify the variations of the cytoskeleton network in morphology by light microscopy when the same cells was extracted with the different extraction above; compared with the low metastasis cells (C7), most of the high metastasis cells (C6) were likely showed that the distribution of the cytoskeleton network was more irregular and uneven as well as gathering on one side to the cell neucleus, and so did a few of Hep G_2 cells (the percentage of regular and even distribution of cytoskeleton, C6: 8.0 ± 1.0; C7: 84.0 ± 2.0; Hep G2: 96.0 ± 2.0; n = 500; χ^2 test, $P < 0.01$). Moreover, extracted with triton X-100 and $(NH_4)_2SO_4$, then labeled by immunoenzymatic technique, the mouse lung adenocarcinoma sublines (C6/C7) were positive for cytokeratin antibody only, but the hepatocellular carcinoma cell (Hep G_2) was positive for both cytokeratin and vimentin antibodies. Besides these, in the same cells, the distribution of the intermediate filament network showed by the immunoenzymatic technique was nearly keeping with that of the cytoskeleton network showed by Coomasie blue R250 stain.

CONCLUSION: (1) It is very difficult to identify the variations of the cytoskeleton network in morphology by light microscopy when the same cell was extracted with triton X-100 and/or $(NH_4)_2SO_4$ then stained with Coomasie blue R250 in comparison. (2) The characterizing distribution of the intermediate filament as well as the cytoskeleton network that was irregular, uneven and gathering on one side to the nucleus in neoplastic cell might provide a valuable information for studying tumor metastasis. (3) In analysing the components of intermediate filament protein of malignant tumor cells, the heterogenous proteins (co-expression) must be taken into consideration.

Key words: Cytoskeleton; Liver neoplasm; Adenocarcinoma; Immunoenzyme technique; Triton X-100; $(NH_4)_2SO_4$; Keratin; Vimentin

Zhang HW. Components and distributions of cytoskeleton network in neoplastic Hep G_2 cells extracted with triton X-100 and $(NH_4)_2SO_4$. *World J Gastroenterol* 2000; 6(Suppl3): 101 Available from: URL: http://www.wjgnet.com/1007-9327/full/v6/iSuppl3/101.htm DOI: http://dx.doi.org/10.3748/wjg.v6.iSuppl3.101

E- Editor: Zhang FF

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