SOFT X-RAY IMAGES OF CHROMOSOMES WITHIN INTACT TUMOR CELLS TREATED WITH COLCHICINE*

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ABSTRACT

In order to avoid any possible effect of separating procedures from intact cell on morphologic structure of the chromosomes, cultivated Hep-2 tumor cells were treated with colchicine and observed by soft X-ray contact microscopy for the first time. The fine structures of chromosomes are more clear with stereo features. The thread-like and coarse granular structures twine and tangle up together within chromosome masses which can not easily be revealed in Wright's stained sample by light microscope or osmium stained sample by transmission electron microscope

Keywords: Soft X-ray contact microscopy Tumor cells Colchicine Chromosomes

1 INTRODUCTION

It has long been an interesting subject for biological and medical workers to study the morphologic structure of chromosomes. Under TEM, chromosomes are observed mainly as dense masses without any more fine structure. Harrison *et al* acquired clear images of lymphocyte chromosomes with sharp stereo features by SEM,[1] but this approach requires isolation of chromosomes which must go through a series of procedures such as hypotonic treatment, fixation and so on, so that the morphologic features of chromosomes in its natural states or within intact cells can not be displayed. Since soft X-ray contact microscopy (SXCM) is suitable for observing wet and thick specimens and the contrast of X-ray image can be provided by the components of biological specimen, thereby avoiding possible artifacts caused by staining and fixation of specimens, it is considered that SXCM is an appropriate tool for studying the fine stuctures of chromosome, especially in its natural states or within intact cells. Manuelidis *et al* studied interphase chromosome by soft X-ray lithography.[2] Shinoharal *et al.*[3] observed separated chromosomes from human

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lymphocytes by SXCM and a fiber with a particle structure in a chromosome was seen. However, up to now, none of report on X-ray image of chromosomes within intact cells is found. In this work, in order to study chromosomes within intact cells, cultured Hep-2 cells were treated with colchicine and observed by SXCM.

2 MATERIALS AND METHODS

The Hep-2 tumor cells at exponential growing stage were treated with colchicine for 15 h. Then the cells were collected and suspensions of 1×10^9 cell/l were prepared. A droplet of the suspension was smeared on Formvar film coated on EM grid. After dried in air, the EM grid was mounted on the film surface of P(MMA-Co-MAA) for soft X-ray exposure. After exposure, the EM grid was removed, the resist was developed in a solution of MIBK and IPA mixture. For SEM observation, a thin layer ($\sim$ 5 nm) of Au was vacuum evaporated onto the developed resist surface at an angle about 15° and followed with a layer ($\sim$ 35 nm) of carbon at normal incidence. For TEM observation, the resist-gold-carbon sandwich was lifted from the glass substrate with 2% HF, picked up on EM grid, and then observed under TEM[4].

3 RESULTS AND DISCUSSIONS

After treated with colchicine, the tumor cells were interrupted at metaphase, nuclear membranes disappeared, chromosomes agglomerated into masses of different

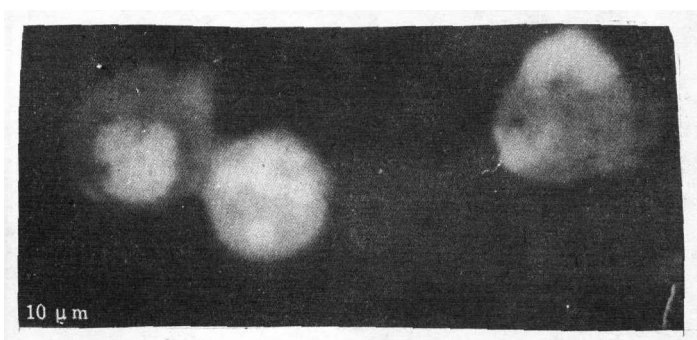

Fig.1 Wright's stained Hep—2 tumor cells treated with colchicine observed by light microscope

sizes and shapes dispersed in cytoplasma (see Fig.1). The fundamental structures of tumor cells treated with colchicine shown by SXCM are similar to that of cell smear observed directly with light microscope (see Fig.2a). However, the X-ray replicas of SEM (Fig.2c) and TEM (Fig.2d) reveal more clearly fine structures. The thread-like and granular structures twine and tangle up in the chromosome masses of intact tumor cells treated with colchicine. As is known to us, these fine stuctures of chromosome masses can not be easily revealed in Wright's stained sample with LM or

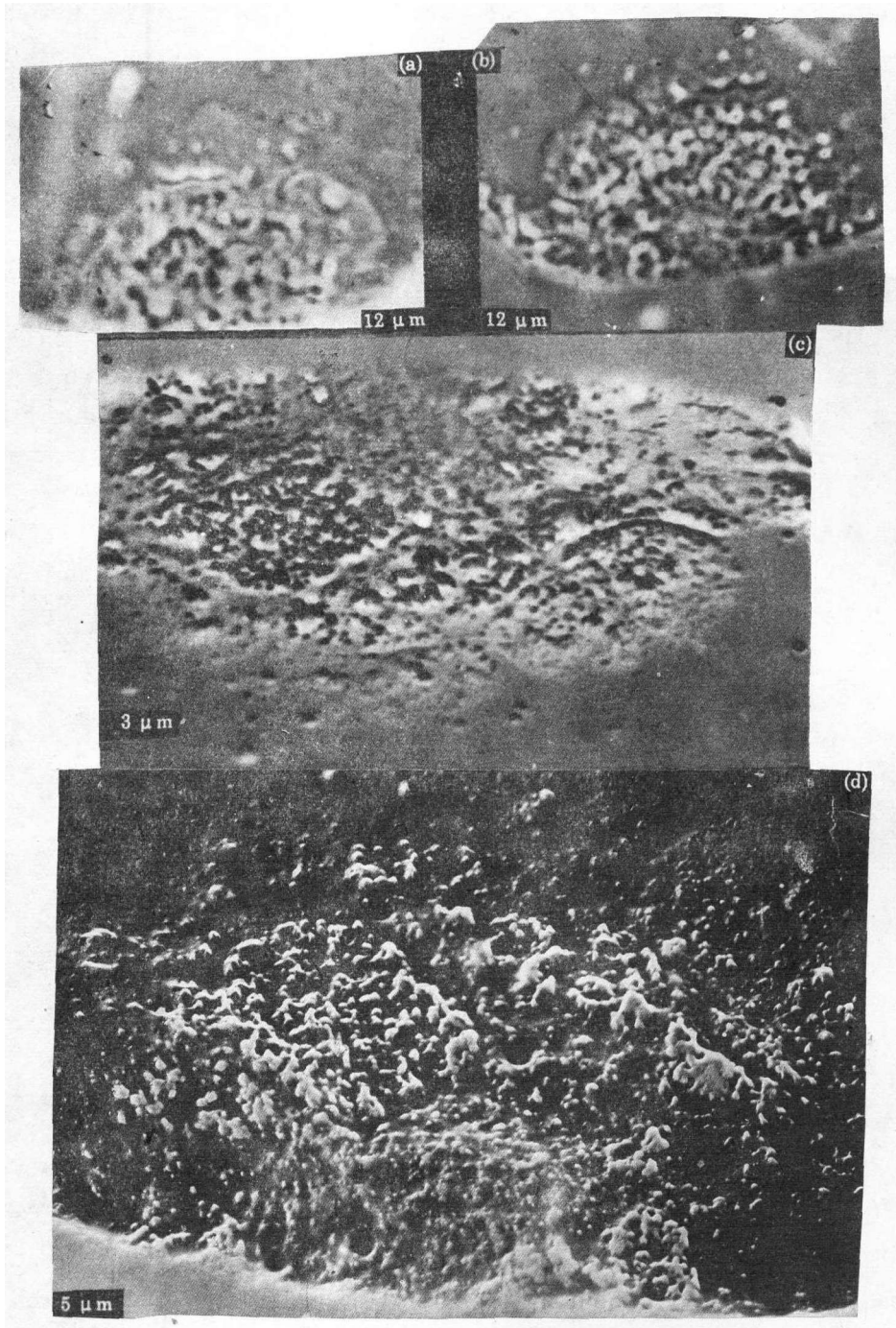

Fig.2 Hep—2 tumor cells treated with colchicine observed at different conditions

(a) original tumor cell smeared on the Formvar coated on EM grid observed by light microscope

(b)X-ray image of the same cell on the resist observed by light microscope

(c) X-ray replica of the same cell observed by SEM (d) X-ray replica of the same cell observed by TEM

osmium stained sample with TEM. These morphological features in chromosome masses resemble that of Harrison's results.[1] The X-ray images of chromosomes show much sharper stereo features than other structures of the cells. Similar phenomenon had been described by Feder[5]. This difference is obvious due to different chemical compositions, and the resulted difference in X-ray absorption. For C-K_α radiation source, it is estimated that there are about e^5 and $e^{2.5}$ more absorption by nucleic acid than by lipids and proteins respectively. This difference is primarily owing to the strong absorption above the L edges of the oxygen and phosphorus atoms abundantly present in nucleic acid[6].

Our results indicate that for observing the morphologic fine structures of chromosome within intact cells. SXCM is a useful tool. In order to avoid any possible effect of colchicine on chromosomes, observation of chromosomes in intact tumor cells without any treatment is being undertaken in our laboratory.

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REFERENCES

[1] Harrison C J, Allen T D. In: Carr K E, Toner P G eds. Cell structure. Churchill Liringstine, 1982, 154.

[2] Manuclids L, Sedat J, Feder R. *Annals of the New York Academy of Science*, 1980, 342:304—325.

[3] Shinohara K, Nakano H, Watanabe M *et al.* In: Sayre D, Howells M, Kirz J *et al.* eds. X-ray microscopy II, Berlin: Springer-Verlag, 1988, 429—432.

[4] Jia Chengzhi, Zhou Zhenghong, Xie Xingshu *et al.* In: Shinohara K, Yada K, Kihara H *et al* eds. X-ray microscopy in biology and medicine. Japan Scientific Societies Press, 1990. 247—250.

[5] Feder R, Spiller E, Topalian J *et al. Science*, 1977, 197:259—269.

[6] McGowan J Wm, Borwein B, Medeiros J A *et al. J Cell Biol*, 1979, 80:732—735.