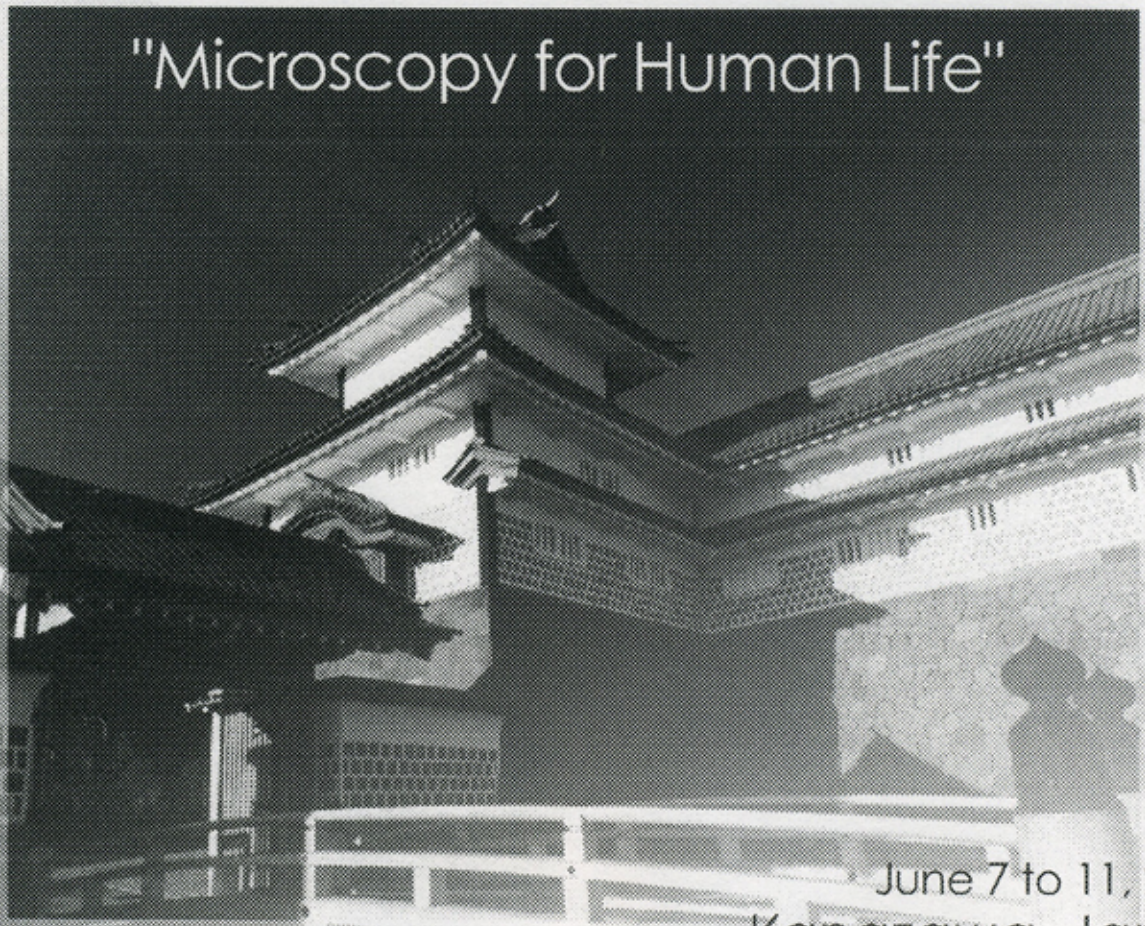
PROCEEDINGS

8th Asia-Pacific Conference on Electron Microscopy (8APEM)

in conjunction with the 60th Annual Meeting of the Japanese Society of Microscopy



June 7 to 11, 2004

Kanazawa, Japan

8th Asia-Pacific Conference on Electron Microscopy (8th APEM)
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8th June 2004

I herein confirm that the following three papers are included in the scientific program of the 8th Asia-Pacific Conference on Electron Microscopy, held in Kanazawa from June 7 to 11, 2004.

1) Paper number 12002

Subcellular damage and Hsp70 accumulation in thermally stressed HEP-G2 cells.

Mirian Strauss, Alegna Rada

2) Paper number 28172

Ultrastructural findings and Hsp70 accumulation in lung and liver alcohol treated rats with and without L-carnitine protection

Noraidys Porras, Marianela Rodriguez, Guillermo Anselmi, Mirian Srauss

3) Paper number 28173

Nucleolar reorganization: stress and tissue-type independent cellular response?

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Nucleolar Reorganization: Stress and Tissue-type Independent Cellular Response?

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Summary

Ultrastructural nucleolar reorganization induced by toxic and thermal stress in different experimental models are studied. Adriamycin-treated cardiac and skeletal cells in vivo as well as embryonic chick heart cells in vitro and thermal stressed human hepatoma cell line showed compact nucleoli, distinct signs of nucleolar alteration and segregation, including a ring-like arrangement of nucleolar components. Stress may cause dynamic and non-permanent changes in the nucleolus which might represent a tissue-type and a stress-type independent cellular response which could be influenced by the presence of protective proteins like Hsp70 through a molecular mechanism that needs to be determined.

Keywords: nucleolar reorganization, adriamycin and thermal stress, cardiac and skeletal muscle, HEP-G2.

The coalescence of the various nucleolar components that morphologically define the highly dynamic nucleolus structure under stress conditions, is not clear. However, nucleolar segregation has been demonstrated in cardiac muscle, skeletal muscle, liver and Novikoff hepatoma ascite cells, following adriamycin (ADR) treatment either in vivo or in vitro [1,2]. With the aim of evaluating if this nucleolar stress reorganization represents both a tissue-type and a stress-type independent cellular response, the present research examined the ultrastructural nucleolar alterations induced by toxic and thermal stresses in different experimental models in vivo and in vitro. Male Sprague-Dawley neonatal rats, were injected i.v. with ADR at two doses of 15 and 25mg/kg and then the animals were killed at 15, 30, 45, 60 min after the toxic treatment. Samples from cardiac and skeletal muscle were analyzed. Besides this, HEP-G2 cells were incubated to 4°C and 40°C for 40min for cold and heat stress respectively. All samples were prepared by ultrastructural standard techniques and subsequently observed in a Hitachi-300 TEM. In addition, cardiac cells from 5 day old chick embryo hearts were cultured on glass coverslips by the hanging-drop technique and treated with ADR (50 μg/ml). These samples were observed by phase-contrast microscopy at 5-60 min.

Controls with trabecular appearance with nucleolar organizer, granular, and fibrillar components randomly distributed were observed (Figure 1: A, B) in the nucleoli of the cardiac and skeletal muscle cells. In ADR-treated samples, pronounced effects were observed at both doses of ADR. These effects included both compact muscle cell nucleoli and distinct signs of nucleolar segregation (Figure 1:C-R), with the segregated granular and fibrillar components in some instances separated from the main portion of the nucleolus, some of them exhibiting a ring-like arrangement (Figure 1: E,H,J,K,P). Changes in the nuclear chromatin were also observed (Figure 1: C,F,J,K,N,Q). Return to normal nucleolar appearance, resembling control samples, was exhibited 60 min. after ADR-treatment (Figure 1: Q,R). The nucleolar subcellular changes were also accompanied by Hsp70 accumulation [3]. All the findings detailed above were also found in the HEP-G2 in vitro cell model with cold and heat stress (Figure 2: B,C). Regarding the embryonic chick heart model, the control samples maintained their normal features for several hours, continuing to pulsate and divide at normal rates (Figure 3A). In contrast, the ADR-treated embryonic heart cells showed nucleolar alteration (Figure 3: B,C). Like the other experimental situations, the nucleolar alterations were not permanent.

Nucleolar segregation is a morphological change of the nucleolus, in which granular components is redistributed, rearranged and separated from the other components accompanied by loss of inter-nucleolonemal spaces. A molecular explanation of the dynamic nucleolar reorganization may be related to the movement of protective proteins like Hsp70 in and out of the nucleolus which has been associated with repair of stress-induced cellular damage by their ability to hold, disaggregate and refold damaged proteins. Nucleolar alteration under stress should be a common step in the control of the quality of protein structure and function in cells, which may represent both a tissue-type and a stress-type independent cellular response.

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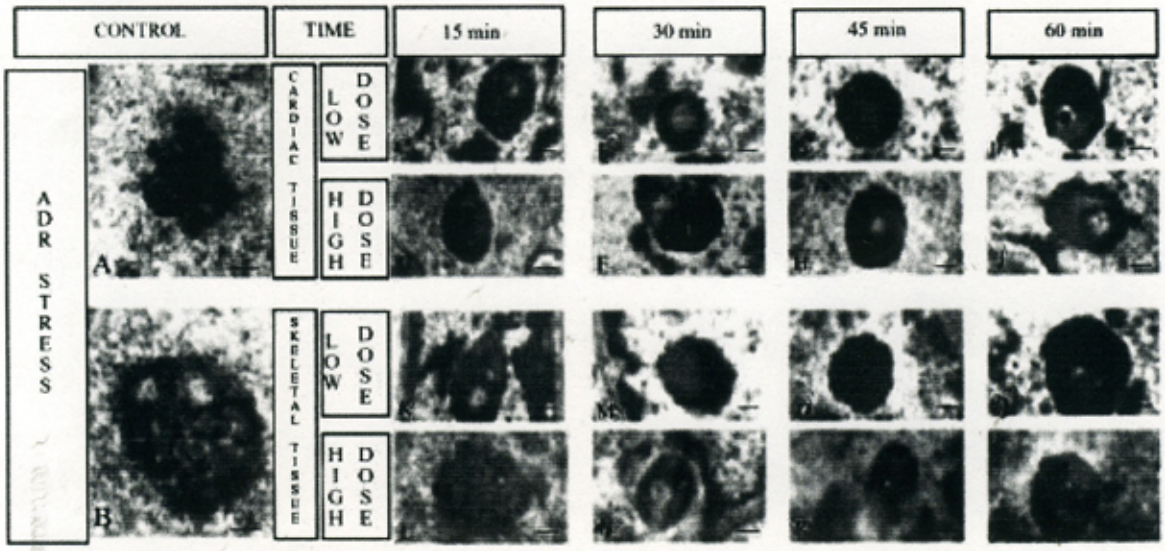

Figure1. TEM of rat cardiac and skeletal tissue nucleolar alterations. A,B: Controls; C-J and K-R: ADR cardiac and skeletal tissues respectively. Bar=0,3µm.

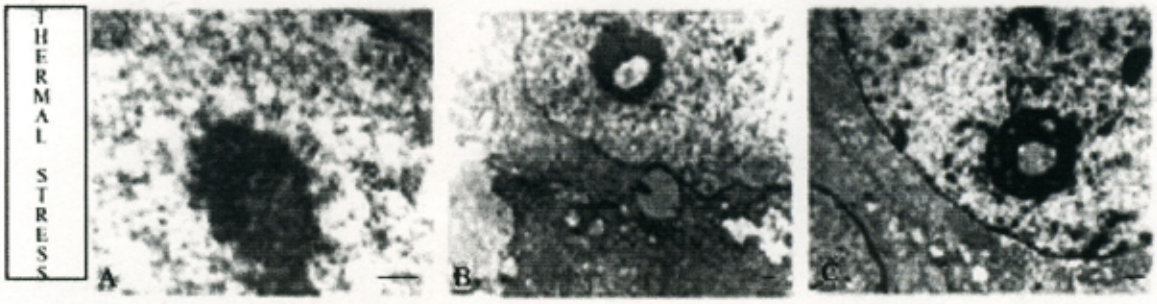

Figure 2. TEM of human hepatoma HEP-G2 cells nucleolar alterations. A: Control, B: Hypothermia and C: Hyperthermia Bar=0,5µm.

Figure 3. Phase-contrast light microscopy of embryonic chick heart cells, A: Control and B,C: ADR treatment. Bar=12,8µm.