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ABSTRACT

An electron-microscope study of x-ray produced HeLa giant cells is described. The results extend earlier light-microscope observations to the submicroscopic region where clear differences from normal structure are apparent. Of particular interest are intranuclear inclusions, nucleolar fragments, membrane abnormalities, and possible mitochondrial changes.
ELECTRON-MICROSCOPE OBSERVATIONS OF RADIATION-INDUCED HE LA GIANTS

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INTRODUCTION

The Hela cell has been a favorite research organism for cellular biologists since its recognition as a stable cell line many years ago. Outstanding work has been done on the x-ray radiation survival of these cells by Puck 1 and on the morphological consequences of radiation by Pomerat. 2 Of particular interest is the production of giant-cell types several days after receiving superlethal doses of radiation. Pomerat and others have carefully studied these cells with phase cinemicrography. However, no electron microscope studies dealing with radiation effects on this cell line have been described. It is the purpose of this report to present preliminary observations on the submicroscopic structure of Hela giants.

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MATERIALS AND METHODS

Cell Technique

Hela S3 cells were routinely grown for many months in 3-oz prescription bottles at 37°C. Twice weekly the cells were generally passed into fresh 199 media containing 10 to 15% lamb serum. For the radiation experiment, each bottle was furnished with a longitudinally split microscope slide during cell passage and allowed to incubate overnight while cell adhesion occurred.

Irradiation was carried out with a 250-kv machine at 15 ma employing 1-mm Al and 1-mm Cu shielding. The dose rate was approximately 70 r/min and was measured by a Victoreen Radocon unit inserted into a dummy 3-oz prescription bottle saturated with water vapor. Doses at 500 and 1000 r were given to various samples. Controls went through the same handling procedure except for the actual irradiation. After irradiation the cells were incubated at 37°C with fresh medium. Medium changes followed at 3-day intervals.

Electron Microscopy

The slides were sampled at 4 and 8 days post irradiation, one of them being stained with H and E for ordinary histology, the other being processed for electron microscopy.

The flat embedding technique suggested by Howatson was employed to obtain sections through the cells parallel to their attachment on the glass slides. The cells were fixed in 1% osmic acid in Veronal buffer for 30 min followed by 15-min dehydrations to absolute ethanol. After treatment with butyl methacrylate monomer, capsules filled with partially polymerized
methacrylate were inverted over selected areas of the slides and placed in a 50°C oven overnight. After polymerization, the capsules were broken off the slides with the aid of solid CO₂ chilling. Although the capsules did not always break off cleanly, the yield was sufficient, since many capsules were placed on each slide. The bases of the capsules were examined in a light microscope and an appropriate area was trimmed for thin sectioning with a Servall Porter Blum Ultramicrotome equipped with a diamond knife. Sections were placed on formvar-coated stainless steel or copper grids and examined in an R.C.A. EMU 2 electron microscope.

EXPERIMENTAL RESULTS

The submicroscopic morphology has been studied by numerous investigators, primarily those interested in virus infection. Figures 1 and 2 represent typical normal cells. Figure 1 shows a low-power over-all view of the organism with its single large nucleus (N), nucleoli (NU), numerous mitochondria (M), microvilli (V) and other cell inclusions. Figure 2 is a high-power micrograph illustrating primarily mitochondrial form. Prominent lipid inclusions (L) and endoplasmic reticulum (ER) are also visible.

The dramatic effects of receiving 1000 r are shown in the survey view of Fig. 3 which was sampled 8 days after the irradiation. Many nuclear or micronuclear fragments (MN) are seen. The region around these nuclei is less electron-dense, giving a halo effect. The amorphous - atrophic region in the center of the cell is of unknown origin. Dense mitochondria are seen, as well as numerous microvilli. Figure 4 shows a similar cell under higher magnification.
Fig. 1. Normal Hela cell displaying a variety of cytoplasmic structure and a typical large nucleus. 14,000X
Fig. 2. Typical mitochondrial profiles in a normal Hela cell. 55,000X
Fig. 3. Survey view of irradiated Hela cell; 1000 r, 8 days postirradiation. 8,000X
Fig. 4. Irradiated Hela cell showing multiple nuclei and nucleolar fragments.
14,000X
The nuclear membranes (NM) have a fuzzy appearance and fragments (F) of presumably nucleolar origin can be seen. Figure 5 illustrates the prominent multiple nucleoli and nucleolar fragments which appear in the giant-cell types. Several rather bizarre cytoplasmic structures (B) are also observable. Figure 6 shows peculiar membranous bodies (MB) within the nucleus of an 8-day post-irradiation (1000 r) cell. It should be noted that intranuclear objects of this sort are commonly seen in these giant cells. Occasional dense membranes (DM) generally within other inclusions are also often observed. Figure 7 shows an unusual spiral-membraned body (S) within a possible micronuclear structure. Several morphologically normal mitochondria as well as the diffuse ribosomal background are included. Figure 8 displays prominent intranuclear fragments and the dense cytoplasmic membranes mentioned earlier. A small golgi region (G) is also observed. The cells sampled earlier -- i.e. 4 days after irradiation -- and those irradiated with lower dosage exhibit a morphology similar to but less exaggerated than that of the 8-day, 1000-r cells.

DISCUSSION

For the purpose of presentation, we can divide the foregoing observations into nuclear and cytoplasmic effects.

Nuclear Changes

It is already well-established that multinucleate forms exist among the giant cells. It is also known that mechanisms such as sister-cell fusion, telo-reduplication, and nuclear fragmentation can produce such multinucleation. Many such cells were seen in the electron microscope.
Fig. 5. Irradiated Hela cell with large multiple nucleoli and nuclear lobes. 14,000X
Fig. 6. Irradiated Hela cell with intranuclear inclusions of a membranous nature. 55,000X
Fig. 7. Irradiated Hela cell having micronuclear structures. 27,000X
Fig. 8. Irradiated Hela cell with intranuclear fragments and cytoplasmic abnormalities. 29,000X
The nuclear membranes often have a "fuzzy" or "thick" appearance and the adjacent cytoplasm seems to contain less material, giving rise to the "halo" effect. Nucleoli are generally very dense and reticular in structure (as in Fig. 5) and occasional fragments are seen which presumably arise from nucleoli. The nuclear ground substance shows no obvious changes, although there is an apparent increase in granularity. Some nuclei show a variety of structures not seen in normal cells. The origin of the peculiar membranous bodies in Fig. 6 is very puzzling. Other nuclei contain vacuoles of apparently cytoplasmic origin. These bizarre elements may be the result of a rather hap-hazard dissolution and reformation of nuclei after a feeble effort toward orderly mitosis in these radiation-injured cells.

**Cytoplasmic effects**

The obvious deviations from normal cytoplasmic morphology seem to be restricted to unusual inclusions of a mitochondrial or micronuclear origin. Figures 6 and 8 included elements that might be interpreted as degenerating mitochondria with occasional very dense lamellar inclusions. Owing to the lipophilic nature of osmic acid, it is not unreasonable to suppose that a slowdown or cessation of β-oxidation in the mitochondria is allowing lipid to accumulate within them. It should be pointed out that mitochondrial changes are difficult to interpret in the Hela cell because of the broad spectrum of normal deviations from classic mitochondrial structure. 6

Numerous areas of morphologically normal endoplasmic reticulum with ribosomal particles were seen in the giant cells together with occasional Golgi regions. The normal cell is endowed with a very active cell membrane sporting many microvilli. This property is undiminished in the giant cells...
as shown in Fig. 1, pointing to pinocytosis as an important physiological activity of these reproductively dead cells.

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REFERENCES


