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Fine-structural study of R-type virus-like particles in a hamster macrophage cell line

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Abstract

In an attempt to isolate virus particles, a hamster macrophage cell line was examined by electron microscopy. This cell line was established from hamster macrophages infiltrating human lymphoid cells heterotransplanted in Syrian hamsters. The cultured cells appeared to be macrophages, containing round smooth nuclei with clear nucleoli, and numerous cytoplasmic primary lysosomes, polysomes, and fragmented rough endoplasmic reticulum (RER). On their surface, many microvillous cytoplasmic processes extended around each cell. Observation at higher magnification revealed the presence of R-type virus-like particles (R-VLPs) in these cells. In the cisternal cavity of RER, R-VLPs were detected at one to several in number in each cavity, and most R-VLPs were detected individually. They had an outer diameter of 90-110nm and contained viral cores with characteristic radial spokes and a smooth viral envelope. Although R-VLPs were detected within RER in a budding pattern, no budding form was found on the cell surface. Neither R-VLPs nor type C virus particles were found in the extracellular space.

The morphology of R-VLPs, with radiating spokes around a viral core, corresponded to that reported in hamster tissues and cells. We found mature ones and budding forms within RER in this hamster macrophage cell line. Notably, hamster macrophages in a cell line established for an extended period of time still contained R-VLPs. This might be evidence for the endogenous nature of R-VLPs in Syrian hamsters, and investigators must be aware of the presence of R-VLPs, when analyze the data on hamster tissues or cells.

Introduction

It has been well-documented that various types of Syrian hamster tissues and cultured cells including brain, liver, lung, spleen, thymus, BHK 21 cell lines and others contain R-type virus-like particles (R-VLPs) [1-10]. These R-VLPs, which have also been called Bernhard-type virus particles [1] or H-type virus particles [9,11], extend peculiar radial spokes around viral cores and contain 60-70sRNA with a molecular weight of 10^7 kd [4]. Although it has not been clearly determined whether these are oncogenic or not [4], it has been reported that their number increased with serial transplantation in vivo or transfer in vitro in hamsters [2,3,5,8,9,11-13]. In fact, numerous R-VLPs were detected in hamster adenovirus-12-induced tumors or other cancers transplanted serially in hamsters [3,11]. In BHK cells, numbers of viruses increased more in vitro than in vivo [14]. It has been proven by molecular studies that R-VLPs are distinct from intracisternal type A virus particles (IAPs) [7,10]. Because of the existence of RNA in R-VLPs, researchers should be aware of its presence in cells or tissues of Syrian hamsters, when analyze the data, as also suggested [4]. Other than cells of hamsters, only rat and bovine cells have been reported to contain R-VLPs [15,16].

We report here an example of cells containing R-VLPs in this study of an established cell line of hamster macrophages.

Materials and Methods

A hamster macrophage cell line was established as reported [17]. In brief, normal human peripheral and umbilical lymphocytes were serially transplanted as tumors in Syrian hamsters. In an attempt to establish a human lymphoid cell line, finely minced tissues of enlarged lymph nodes from these hamsters were cultured, and finally a macrophage cell line, derived from human tumor-infiltrating macrophages of hamster origin was established after 29 months. For electron-microscopic study, the cultured cells of a hamster macrophage cell line were centrifuged at 1000rpm for 3 min., and the cell pellets were fixed in 3% glutaraldehyde and postfixed with 1% OsO_4 solution and dehydrated through a graded ethanol series as described previously [18-20]. Then, after passage through propylene oxide, hardening of resin (Luveak 812, Nacalai Tesque, Kyoto, Japan) at 60°C for two nights was performed, and trimming was performed after examination by

inverted microscopy. Ultrathin sections were cut with a diamond knife, and stained with both uranyl acetate and lead citrate solutions. The ultrathin sections were observed with an Hitachi H-300 type electron microscope (Hitachi Co. Ltd., Tokyo Japan), magnified at X3,000 - X40,000.

Results

The general features of cultured cells were compatible with those of macrophages (Fig. 1). Heterochromatin-rich smooth round nuclei and peculiar nucleoli were found,

and in the cytoplasm fragmented rough endoplasmic reticulum (RER) and many primary lysosomes as well as many polysomes were present (Fig. 2). Microfilaments and microtubules were also found in the cytoplasm. On cell surfaces, many long-slender fine cytoplasmic processes, occasionally exhibiting branching, extended around these cells (Fig. 1, inset; Fig. 2). Pinocytotic vesicles were sometimes found along the plasma membranes

Peculiar findings included the presence of R-VLPs within RER cavities (Figs. 3-5). These RER cavities were very close to mitochondria in the cytoplasm, resembling the formation of RER-mitochondrial complexes (Figs. 3,4). R-VLPs were composed of viral cores exhibiting a radiating pattern of spokes with smooth viral envelope of unit membrane. Doughnut-shaped cores associated with radial spokes were also found on occasion, in addition to dense ones (Fig. 5). Their sizes were approximately 90-110 nm in diameter, and one to several particles were present in each cisternal cavity (Figs. 3-5). Budding particles were also detected on the surface of the RER (Fig. 4), but no beaded structures were found. Some R-VLPs appeared to be located in the cytoplasm, based on the direction of cutting of ultrathin sections (Fig. 3). In addition to the core with peculiar radiating spokes, the characteristic findings

of these R-VLPs included intracisternal localization of RER with budding forms, which were exclusively intracisternal (Figs. 3-5). No budding particles were detected on cell surfaces. Neither R-VLPs nor type C virus particles were found in the extracellular spaces in this cell line.

Discussion

The characterization of this hamster cell line as macrophages was reported previously, based on morphological and enzymatic evidence [17]. The morphological findings for R-VLPs detected in this hamster cell line were identical to those reported previously [1,3,4,5,9,11]. In addition to the characteristic radial spokes from the core, these viruses contained 60-70s RNA with a molecular weight of 10^7 kd [4]. Many tissues and cells of Syrian hamsters have been reported to contain this type of virus, including BHK-21 cells and their derivatives [1-14], and other than cells of hamsters only bovine and rat cells have been reported to contain R-VLPs [15,16]. It was reported that, in BHK-21 cells, production of R-VLPs was enhanced following treatment with carcinogenic agents [9] or dexamethasone [6], but that R-VLPs decreased in number following treatment with actinomycin D [6]. In hamster hepatoma cells, dibutyryl cyclic AMP and theophylline also increased numbers of R-VLPs in vitro [5]. These findings suggest that processes of transcription are required for the production of R-VLPs [6]. In addition, no study has demonstrated the oncogenicity and/or infectivity of R-VLPs in vitro or in vivo.

As reported in the literature, cells of adenovirus-12-induced tumors, especially when transplanted serially in Syrian hamsters, contained many R-VLPs, as revealed by electron microscopy [1,3,12]. The number of R-VLPs increased extraordinarily with serial transplantation in vivo and in vitro [3,5,9], with beaded structures found at sites of budding [3]. These are unusual patterns of viral budding, and suggest the presence of special budding sites on the surface of RER [3]. In the present study, because of the small numbers of R-VLPs, no such budding patterns were found in the hamster cell line examined.

The present findings and those of previous studies suggest that the R-VLPs are endogenous to Syrian hamsters. Researchers must thus bear in mind the existence of these R-VLPs in hamster tissues and cells, especially those of Syrian hamsters, when performing molecular studies.

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