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Cell Proliferation and Objects Such as Fig Leaf and Chrysanthemum Generated in Agar Cultures of Egg-Prepared DNA (Hepg2 Cells) Crown Cells with Monolaurin

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Abstract

Synthesized DNA crown cells (artificial cells) capable of proliferating within egg white in vivo can be prepared in vitro using sphingosine (Sph)-DNA-adenosine-monolaurin compounds. In previous studies using synthetic DNA (E. coli, human placenta, ascidian, Streptomyces) crown cells cultured with monolaurin or egg white, the proliferation of objects similar in appearance to thallus, double cells, morning glory, and cell ring-like objects were observed on agar plates. Whether similar objects could be formed by DNA crown cells prepared with egg (egg-prepared DNA crown cells) remains unclear. The present study used egg-prepared DNA crown cells (HepG2 cells) and monolaurin to generate various object-like formations by cells proliferating on agar plates. Here, we describe the microscopic characteristics of cell formations resembling objects such as leaves

Keywords: Egg prepared DNA (HepG2 cells) crown cells; Agar plate cultures; Sphingosine-DNA; Objects like fig leaf; Monolaurin

Introduction

Artificial cells covered with DNA are referred to as DNA crown cells [1-3]. Synthetic DNA crown cells can develop into DNA crown cells in culture within egg white and prepared using sphingosine (Sph)-DNA and adenosine-monolaurin (A-M) compounds. In previous studies, various types of cell proliferation were observed and demonstrated microscopically when monolaurin-treated synthetic DNA (*E. coli*, human placenta, ascidian, *Streptomyces*) crown cells were cultivated with egg white on agar plates [4-8]. However, whether similar objects can be observed in cultures of DNA crown cells prepared with eggs (egg-prepared DNA crown cells) remains unclear. In the present study, DNA crown cells were first prepared using egg and DNA from HepG2 carcinoma tumor cells. The shapes formed by proliferating cells were then observed after culture of the DNA (HepG2 cell) crown cells with monolaurin on agar plates. Many types of object shapes and cell proliferation patterns were observed. Several proliferation patterns and objects, such as fig leaves and chrysanthemums, were observed under microscopy.

Materials and Methods

Materials

The materials used in the present study were the same as those employed in previous studies [9,10]: Sph (Tokyo Kasei, Japan), DNA (from HepG2 cells), adenosine (Sigma-Aldrich; Wako, Japan), monolaurin (Tokyo Kasei), and A-M, synthesized from a mixture of adenosine and monolaurin [9,10]. Monolaurin solutions were prepared to a final concentration of 0.1 M in distilled water. Agar plates prepared with standard agar medium (SMA) (AS ONE, Japan) were used. Eggs were obtained from a local market.

Methods

Preparation of synthetic DNA (HepG2) crown cells

Synthetic DNA (HepG2) crown cells were prepared as described previously [9,10]. Briefly, 180 µL of Sph (10 mM) and 50 µL of DNA (0.3 µg/µL) were combined, and the mixture was heated and cooled twice. A-M solution (100 µL) was added, and

the mixture was incubated at 37°C for 15 min. Next, 30 µL of monolaurin solution was added, and the mixture was incubated at 37°C for another 5 min. The resulting suspension was used as synthetic DNA (HepG2) crown cells.

Preparation of DNA (HepG2) crown cells using egg:

i. Two eggs were prepared, and approximately 0.2-0.3 mL of sample (synthetic DNA [HepG2] crown cells) was injected within each egg white.

ii. The eggs were then incubated for 7 days at 37°C.

iii. The egg white was then collected from the eggs and kept as a sample (egg-prepared DNA [HepG2] crown cells) in the freezer.

Culture of monolaurin with the sample on agar plates was performed as follows:

i. Sample (1.5 mL) was plated on the whole surface of three agar plates, and the upper fluid was removed.

ii. Immediately, 1.5 mL of twice-diluted 0.1 M monolaurin was poured onto each agar plate.

iii. After removing excess monolaurin, the plates were inverted and incubated for 7 days at 37°C and observed at 5 and 7 days.

iv. After 7 days of incubation, the plates were stored at 4°C.

Microscopic observations

Objects on plates were observed directly under a light microscope and with the naked eye.

Results and Discussion

Figure 1 shows the microscopic appearance of DNA (HepG2) crown cells recovered from egg white. Experiments on cell recovery were carried out to determine whether cells were present in egg white injected with synthetic DNA (HepG2) crown cells and then incubated. Cell recovery experiments were carried out as described previously [9,10], using Dulbecco's minimal essential Eagles medium supplemented with 10% bovine serum. Many cells were observed, including cells that formed a ring-like arrangement or structure (arrows a, b, and c). The approximate size of these cells was 7-8 µm (arrow b). Figure 2 shows a photograph of an agar plate (Plate 3) of egg-prepared DNA (HepG2) crown cells after 7 days of culture with monolaurin. A cloudy region (arrow), visible to the naked eye, was widespread in the Petri dish, with a diameter of 8.0 cm. Figure 3 shows the microscopic appearance of the egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with monolaurin. Numerous round patterns and shapes of various sizes were observed (arrows a, b, and c). The approximate size of the objects (arrow b) was 7-8 µm. Figure 4 shows the microscopic appearance of the egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with monolaurin. Many round, even-shaped structures were observed (arrows a and b). The size (arrow a) of the structures was approximately 21-24 µm. Figure 5 shows the microscopic appearance of the egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with egg white. Numerous objects of various sizes and irregular shapes were observed (arrows a, b, and c). These objects were composed of many cells. Also, large objects with a bar-like appearance were observed (arrow d). The approximate size was of these objects was 4-5 µm (arrow c).

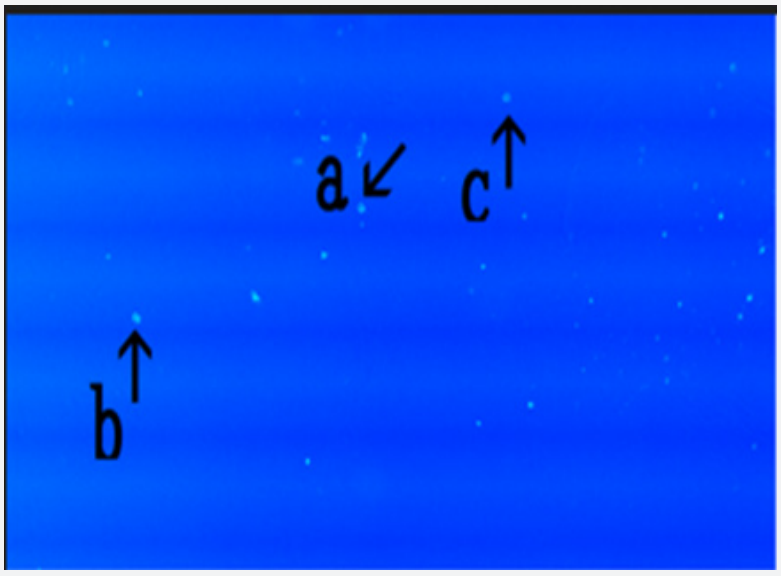

Figure 1: Microscopic appearance of egg-prepared DNA (HepG2) crown cells recovered from egg white. Many cells were observed (arrows a, b, and c), and the approximate size was 7-8 µm (arrow b).

d) NKX3.1 staining shows strong nuclear staining in the atypical epithelioid cells

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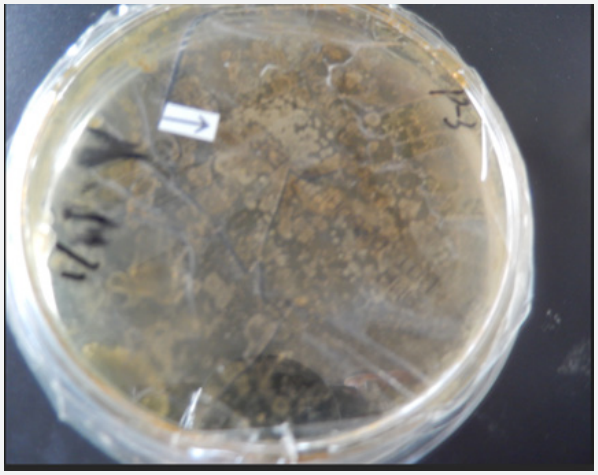

Figure 2: Photograph of agar plate (Plate 3) of egg-prepared DNA (HepG2) crown cells after 7 days of culture with monolaurin. A cloudy region (arrow) visible to the naked eye was prominent.

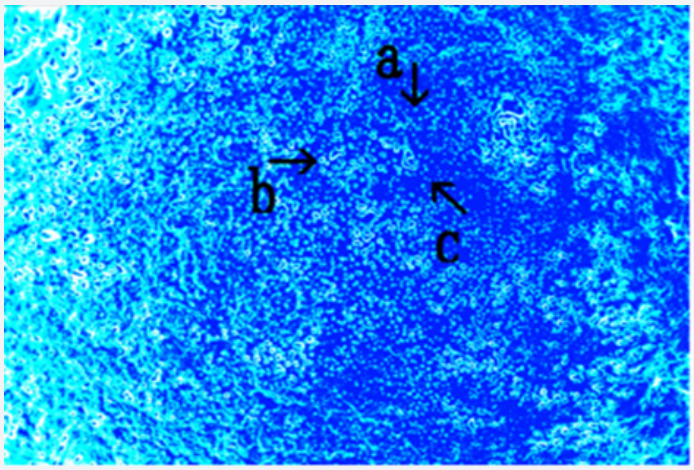

Figure 3: Microscopic appearance of egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with monolaurin. Many objects were observed (arrows a, b, and c), and the approximate size of the object (arrow b) was 7-8 μm.

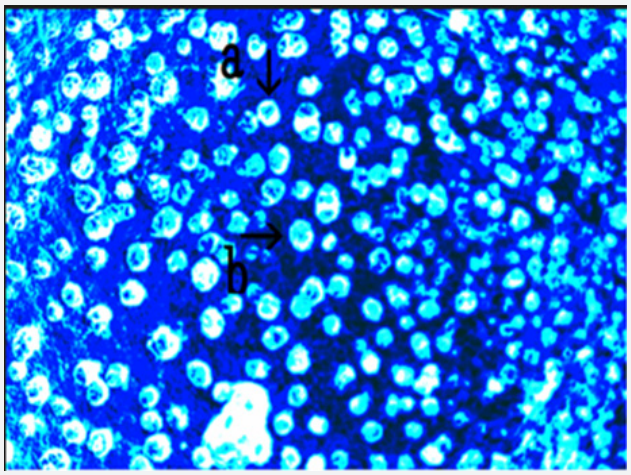

Figure 4: Microscopic appearance of egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with monolaurin. Many evenly spaced, similarly shaped objects were observed (arrows a and b), and the size (arrow a) was approximately 21-24 μm.

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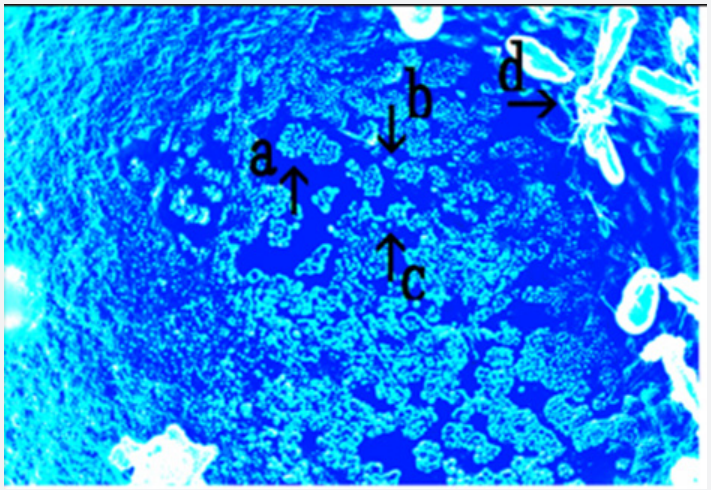

Figure 5: Microscopic appearance of egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with egg white. Many objects were observed (arrows a, b, and c), and the approximate size was 4-5 μm (arrow c).

Figure 6 shows the microscopic appearance of the egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with monolaurin. Many chrysanthemum-like objects were observed (arrows a and b). The approximate diameter of the object (arrow a) was 56-60 μm. Figure 7 shows the microscopic appearance of egg-prepared DNA (HepG2) crown cells from Plate 2 (plate not shown) after 7 days of culture with monolaurin. Objects resembling fig leaves were observed (arrows a, b, and c). The approximate diameter of the object was 42-45 μm (arrow d). Figure 8 shows the microscopic appearance of egg-prepared DNA (HepG2) crown cells on Plate 1 (plate not shown) after 7 days of culture with monolaurin. Many chrysanthemum-like objects of varying size were observed (arrows a and b). Also, the space between the objects exhibited a network-like structure (arrow c). Objects potentially associated with the network-like structures were also observed (arrows d and e). The approximate size of these structures was 28-30 μm (arrow a) and 14-16 μm (arrow b).

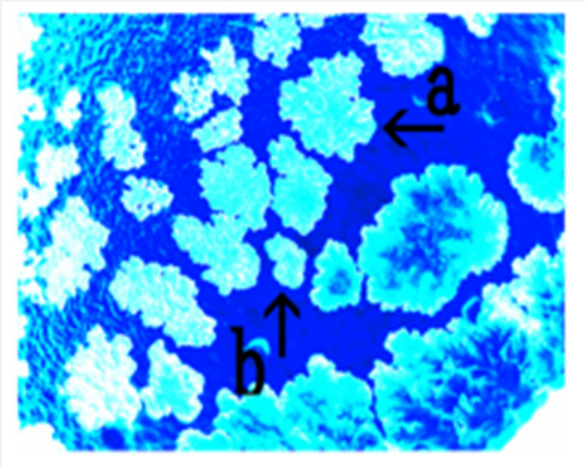

Figure 6: Microscopic appearance of egg-prepared DNA (HepG2) crown cells in Figure 2 after 7 days of culture with monolaurin. Many chrysanthemum-like objects were observed (arrows a and b), and the approximate diameter (arrow a) was 56-60 μm.

In previous experiments using synthetic DNA (*E. coli*, human placenta, ascidian, *Streptomyces*) crown cells, objects with features such as tallus, double cells, morning glory and cell rings were observed on the agar plates [4-8]. The present experiments examined whether similar objects are formed in agar cultures of egg-prepared DNA (HepG2) crown cells cultured with monolaurin.

Many objects and cells of varying size or homogeneous sizes were observed (Figure 3, arrows a, b, and c; Figure 4 arrows a and b). Also, objects composed of small cells (Figure 5 arrow c) were observed (Figure 5 arrows a and b). These cells exhibited characteristics of proliferating cells, but the mechanism of this proliferation may differ, as suggested by differences in the size

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or shape of the objects observed in Figures 3 through 5. At least three types of cell proliferation were observed, but it remains unclear how the cells proliferates. Proliferation could depend on the characteristics of the cells and whether basic cells are present. For example, cells that readily divide or grow could form the population that generates the objects (Figure 3 for dividing cells and Figure 4 for growing cells). These cells might also readily adsorb to one another (Figure 5). Although objects such as leas tips and chrysanthemum were observed (Figure 6 arrows a and b; Figure 7 arrows a, b, and c), it remains unclear how these objects were formed. Though clear evidence was not obtained in this study, the results could provide hints regarding the mechanism of object formation (Figure 8).

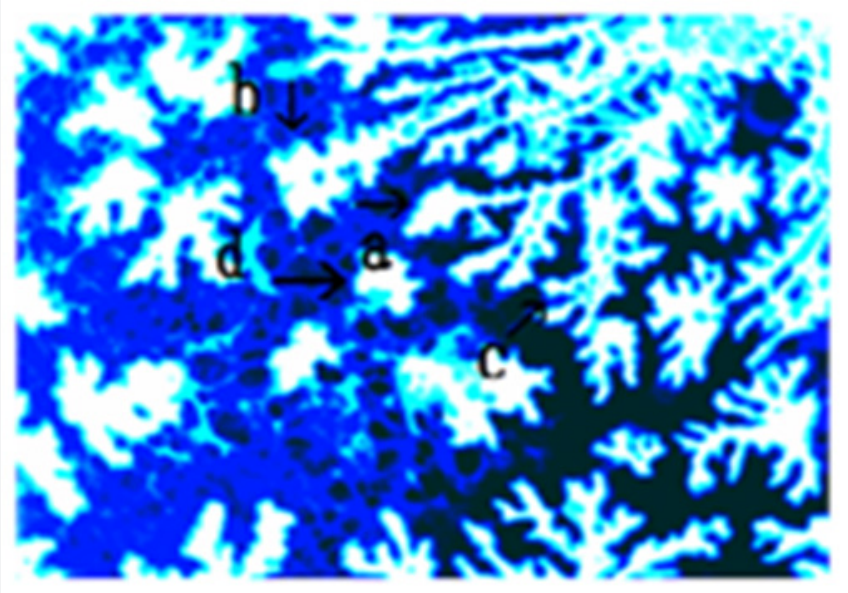

Figure 7: Microscopic appearance of egg-prepared DNA (HepG2) crown cells in Plate 2 (plate not shown) after 7 days of culture with monolaurin. Objects like fig leaves were observed (arrows a, b, and c), and the approximate diameter was 42-45 μm (arrow d).

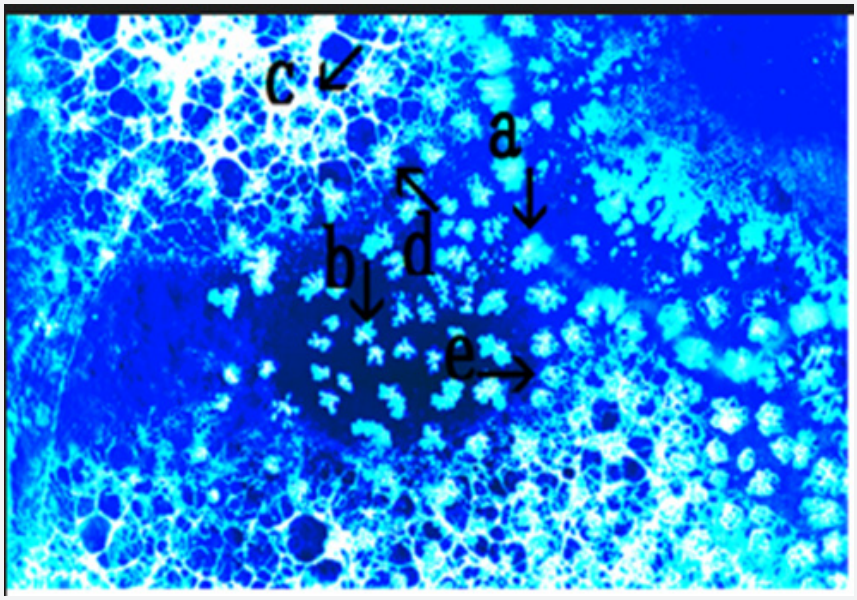

Figure 8: Microscopic appearance of egg-prepared DNA (HepG2) crown cells in Plate 1 (plate not shown) after 7 days of culture with monolaurin. Many objects were observed (arrows a and b). The spaces between objects exhibited a network-like appearance (arrow c). Objects within the network-like structure were also observed (arrows d and e). The approximate size was 28-30 μm for arrow a and 14-16 μm for arrow b.

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Many objects such as chrysanthemum flowers of various sizes were observed (Figure 8 arrows a and b). A large area of net-like objects was also observed (Figure 8 arrow c). Objects that could serve as the components of these net-like objects were also observed (Fig. 8 arrows d and e). It is thought that the objects observed in Figure 8 (arrows a, b, d, and e) were derived from the net-like objects (Figure 8c). If this assumption is correct, it represents a new finding with regard to the mechanism by which cells proliferate. Previous studies on the assembly [11-13] or cultivation [14-17] of cells demonstrated that cells proliferate from the assembly of synthetic DNA crown cells or from elongation of the cell membrane, which consists of Sph-DNA-adenosine-monolaurin.

On the other hand, in the present study, objects like nets were formed first, and objects like cells were formed based on the lattice of these net-like objects. That is, objects corresponding to the size or shape of the lattice may be formed. Most of the proliferating cells were round in shape and at most 20 μm in size. However, cells that proliferate according to a different mechanism could form the irregular shapes we observed (for example, quadrilaterals or triangles) or large size (>20 μm). Present results clearly indicate that the net-like objects were formed from Sph-DNA-adenosine-monolaurin and might contain the components of egg white, suggesting that the objects (Figure 8 a, b, d, and e) are constructed of these components. As such objects have not been observed when combining other types of synthetic DNA crown cells and monolaurin, it is not clear whether they exhibit characteristics similar to cancer cells.

On the other hand, wide space could be seen with the naked eye (Figure 2), and many types of objects and numerous indicators of cell proliferation could be observed within these spaces. The objects were observed within limited spaces, and thus, it was difficult to observe all aspects of the objects. Objects exhibiting characteristic features were formed using synthetic DNA (*E. coli*, human placenta, ascidian, and *Streptomyces*) crown cells, and in this study, objects exhibiting new features were observed. These data suggest that particular objects can be formed using synthetic DNA crown cells.

The objects observed in the present study could benefit research on DNA crown cells as well as synthetic DNA crown cells and egg-prepared DNA crown cells. However, it is important to demonstrate that the objects are living. The present study demonstrated that characteristic objects were formed using five kinds of synthetic or DNA crown cells: synthetic DNA (*E. coli*, human placenta, ascidian, and *Streptomyces*) and egg-prepared DNA (HepG2) crown cells. The results indicate that synthetic DNA crown cells and egg-prepared DNA crown cells may form characteristic objects in response to DNA consisting of crown cells. Synthetic DNA (HepG2) crown cells can be cultivated in vitro to establish cell strains. Therefore, the objects that grew in the Petri dishes can be assumed to be living. It is important that such objects can be formed using cells cultured on agar medium. Future studies will examine whether the objects visible to the naked eye in the Petri dishes (Figure 2 in present paper, 4-8) were indeed living.

Two primary objects were introduced in the present paper:

i. objects like a fig leaf, Human cancer (HepG2) EDCCM (FL);

ii. objects like a chrysanthemum, Human cancer (HepG2) EDCCM (Chr).

HepG2 (name of DNA source), where EDCC refers to egg-prepared DNA crown cells, M refers to monolaurin, and fig leaf (FL) and chrysanthemum (Chr) refer to the characteristic structure.

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