Analysis of cell fusion induced by EB virus growth in epithelial cells

上皮系細胞におけるＥＢウィルス増殖による細胞融合機構の解析

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研究発表

1. Sato, H., Kida, Y., Mai, M., Endo, Y., Sasaki, T., Tanaka, J., and Seiki, M. Expression of genes encoding type IV collagen-degrading metalloproteinases and their inhibitor TIMPs in various human tumor cells. Oncogene (in press)

2. Yoshizaki, T., Takimoto, T., Takeshita, H., Tanaka, S., Furukawa, M., Seikiki, M., and Sato, H. Epstein-Barr virus lytic cycle spreads via cell fusion in a nasopharyngeal carcinoma hybrid cell line. (submitted for publication)

3. Sato, H., Takeshita, H., Furukawa, M., and Seiki, M. Epstein-Barr virus BZLF1 transactivator is a negative regulator of Jun. (submitted for publication)

研究の目的

ＥＢウイルスのＢＺＬＦ１にコードされる１１０キロダルトンの糖蛋白は単純ヘルペスウイルスの細胞融合活性を担うｇＢ蛋白とのアミノ酸配列に高い類似性があることよりＥＢウイルスもまた単純ヘルペスウイルスの様な細胞融合活性を持つことが予想されたが、ＥＢウイルスにはｌｙｔｉｃな感染系又はウイルス高産生性細胞が知られていないためＥＢウイルス増殖による細胞融合は実験的には証明されていなかった。我々は上咽頭癌由来上皮系細胞株ＮＰＣ－ＫＴのサブクローンｃｌ．Ｓ６１細胞を用いてＥＢウイルスによる細胞融合、封入体形成をはじめて実験的に証明した。一方、ＥＢウイルスはバーキットリンフォーマ、伝染性単核症のほかに、上咽頭癌、mid facial T cell lymphoma, oral hairly leukoplakia、などＥＢウイルスレセプターであるＣＲ２の存在しない細胞への感染、発症にも関与していることが明かとなったがその感染機構は不明である。ｃｌ．Ｓ６１細胞はＣＲ２を有しないにもかかわらず高頻度に細胞融合を起こすことから、他のウイルスレセプター陰性の細胞との細胞融合が予想され、従来提唱されていたＥＢウイルスの細胞融合によるレセプター陰性細胞へのｃｅｌｌ　ｔｏ　ｃｅｌｌによる感染の可能性が示唆される。本研究ではｃｌ．Ｓ６１細胞のＥＢウイルス陰性細胞特にＣＲ２陰性細胞との細胞融合の可能性を検討しこのメカニズムによるＥＢウイルス感染の可能性を探る。

研究成果

ABSTRACT

NPC-KT cl.S61, a subclone derived from an epithelial-nasopharyngeal carcinoma hybrid cell line NPC-KT, showed extensive Epstein-Barr virus (EBV) production and cytophapthic changes characteristic of herpesvirus replication, including formation of multinucleated giant cells and inclusion bodies, when EBV replicative cycle was induced by 5-iodo-2'-deoxyuridine. Syncytia formation of cl.S61 cells was inhibited by 2-deoxyglucose and Acyclovir, inhibitors of glycosylation and EBV DNA polymerase, respectively, with a concomitant decrease in the number of cells expressing EBV growth-associated antigens to the level of parental NPC-KT cells, which did not form syncytia during virus synthesis. However, the frequency of virus antigen expression in NPC-KT cells was not significantly affected. These results suggest that the EBV replicative cycle spreads via cell fusion in cl.S61 cells. It was also demonstrated by in situ autoradiography that cl.S61 cells producing virus fused to not only EBV receptor/CR2 positive Raji and BJAB cells but also

to receptor negative Jurkat cells. The possible mechanism of entry of EBV into cells devoid of virus recptor by cell fusion is discussed.

Epstein-Barr virus (EBV), a human herpes virus, is widespread in all human communities. Infection with the virus usually results in either subclinical disease or infectious mononucleosis (1). The virus is also associated with Burkitt's lymphoma and can transform B lymphocytes in vitro, so it is generally considered to be a lymphotropic virus (2). The detection of EBV-associated nuclear antigen (EBNA) and viral DNA in nasopharyngeal carcinoma (NPC) have revealed that EBV can infect epithelial cells and is associated with their transformation (3). The detection of viral antigens including EBNA, early antigen (EA)/ capsid antigen (VCA) and viral DNA in epithelial cells suggested that epithelial cells of the oropharynx were the sites of virus replication in vivo (4,5). Although Young et al. detected EBV receptor/CR2 in pharyngeal epithelial cells in a cell

differentiation-dependent manner (6), raising the possibility of direct virus entry into naturally exposed epithelium, infection of epithelial cells with EBV has not been well explained. In oral hairy leukoplakia in human immunodeficiency virus-infected patients, EBV permissively infects and replicates actively in epithelial cells of the tongue followed by the clinical appearence of oral hairy leukoplakia (7). Thus, EBV replication in epithelial cells produces lytic infection in vivo. However, viral replication in epithelial cells has not been examined in vitro due to the lack of an appropriate tissue culture system.

An EBV-carrying epithelial-NPC hybrid cell line (NPC-KT) was established by fusing primary NPC epithelial cells with an epithelial cell line derived from human adenoid tissue (8). EBV from NPC-KT cells lacking defective viral DNA molecules has dual properties of transforming human B lymphocytes and of superinfecting Raji cells, inducing EA and viral DNA synthesis (8,9,10,11). An EBV-high producer subclone of NPC-KT cells, cl.S61, showed cytopathic changes characteristic of

herpesvirus replication, including formation of multinucleated giant cells and inclusion bodies, when the virus replicative cycle was induced by 5'-iodo-2'-deoxyuridine (IdUR) (12). EBV replication in cl.S61 cells after IdUR treatemnt was very effective compared to parental NPC-KT cells and other EBV producer B cell lines including P3HR-1 and B95-8 cells. To analyze how EBV replicates so effectively in cl.S61 cells, the effect of 2-deoxyglucose and Acyclovir (ACV), both of which inhibit syncytia formation of cl.S61 cells (12), on induction of viral replicative cycle was compared between cl.S61 and NPC-KT cells. NPC-KT and cl.S61 cells were treated with IdUR in the presence or absence of 2-deoxyglucose or ACV, then stained for EA/VCA with the serum from a patient with NPC as described previously (12). More than 90 % of nuclei of cl.S61 cells treated with IdUR were positively stained by indirect immunostaining with the NPC patient serum. On the contrary, only 31.2 % of NPC-KT cell nuclei were positively stained (Table 1). As shown in Table 1 and in a previous report, 2-deoxyglucose and ACV inhibited syncytia formation

Table 1. Effect of ACV and 2-deoxyglucose on EA/VCA expression of cl.S61 and NPC-KT cells.

Cells	Culture addition	No. of syncytia/ 1,000 cells[a] (% inhibition)	EA/VCA positive nuclei[b] (%) (% inhibition)
cl.S61	None	112 (-)	91.3 (-)
	ACV[c]	0 (100)	28.6 (68.7)
	2-Deoxyglucose(1mM)[d]	4 (96.4)	33.5 (63.3)
	2-Deoxyglucose(10mM)[d]	1 (99.1)	30.8 (66.3)
NPC-KT	None	0 (-)	31.2 (-)
	ACV[c]	0 (-)	30.5 (2.2)
	2-Deoxyglucose(1 mM)[d]	0 (-)	29.5 (5.4)
	2-Deoxyglucose(10 mM)[d]	0 (-)	27.6 (11.5)

[a]Number of cells with more than three nuclei per 1,000 cells was counted 2 days after removal of IdUR.

[b]Cells were stained with serum from a patient with NPC (EA antibody titer, 1:160; VCA antibody titer, 1:640) by indirect immunofluorescence.

[c]ACV (100 μM) was included in the culture throughout IdUR induction.

[d]2-Deoxyglucose (1 or 10 mM) was included in the culture after removal of IdUR.

in cl.S61 cells treated with IdUR (12). Induction of EA/VCA expression by IdUR treatment in NPC-KT cells, which did not form multinucleated cells after IdUR treatment, was unaffected by 2-deoxyglucose and ACV. Although ACV inhibited synthesis of virus-associated proteins belonging to the late group (12), the number of NPC-KT cells positively stained with NPC patient serum was not significantly affected. However, EA/VCA-expressing cl.S61 cells decreased to the level of parental NPC-KT cells in the presence of 2-deoxyglucose and ACV, which interfered with syncytia the formation of cl.S61 cells treated with IdUR. Reduction of virus replication in cl.S61 but not in NPC-KT cells by 2-deoxyglucose, was also confirmed by Southern hybridization which detected viral DNA (data not shown). Cell fusion occurs between infected and uninfected cells in herpes simplex virus infection (13), and it has been shown previously that in multinucleated cl.S61 cells, most of the nuclei were positively stained for EBV antigens with NPC patient serum (12). These results suggest that the EBV replicative cycle spreads to secondary cells

via cell fusion which takes place only in cl.S61 but not in NPC-KT cells. The EBV lytic cycle is known to be induced by the expression of viral immediate-early genes including BZLF1 (14), and these factors may spread to the surrounding cells via cell fusion in cl.S61 cells.

The cl.S61 cells do not have EBV receptor/CR2 on their surface indicating that virus receptor is not essential for cell fusion induced by virus replication. Since cell fusion may be the mode of EBV infection in cells lacking the EBV receptor, the next issue is whether EBV infection can spread from cl.S61 to secondary cells other than cl.S61 cells, which are EBV receptor negative. We therefore investigated whether cl.S61 cells producing virus can fuse to cells which are either virus receptor positive or negative. Raji (an EBV genome positive and virus receptor/CR2 positive B-cell line), BJAB (an EBV genome negative and EBV receptor/CR2 positive B-cell line), and Jurkat, an EBV genome negative and EBV receptor/CR2 negative T-cell line, were cultured in RPMI1640 medium supplemented with 5% FCS containing 10 μCi/ml [^{3}H]-

thymidine for 48 h, before cocultivation with IdUR-treated cl.S61 cells. cl.S61 cells were treated with IdUR on cover slips, and were then cocultured with 10^5/ml lymphoblastoid cells labeled with [^{3}H]-thymidine for 2 days. Cells were washed with PBS and fixed with ethanol for 30 min, coated with Sakura NR-M2 emulsion and exposed for 2 weeks at 4°. Cover slips were then developed, stained with Giemsa and examined microscopically. More than 95% of lymphoblastoid cell nuclei labeled with [^{3}H]-thymidine were positively detected under these conditions. Raji cells formed aggregations around cl.S61 cells treated with IdUR within an hour after cocultivation (Fig. 1A), but not around untreated cells (data not shown). Raji cell nuclei labeled with [^{3}H]-thymidine were frequently identified in multinucleated giant cells by in situ autoradiography indicating that Raji cells fuse to cl.S61 cells treated with IdUR (Fig.1B). BJAB cells formed aggregations less frequently around cl.S61 cells (data not shown) and BJAB cell nuclei were also identified in multinucleated cl.S61 cells with a lower frequency than Raji nuclei (Fig.1C). Jurkat

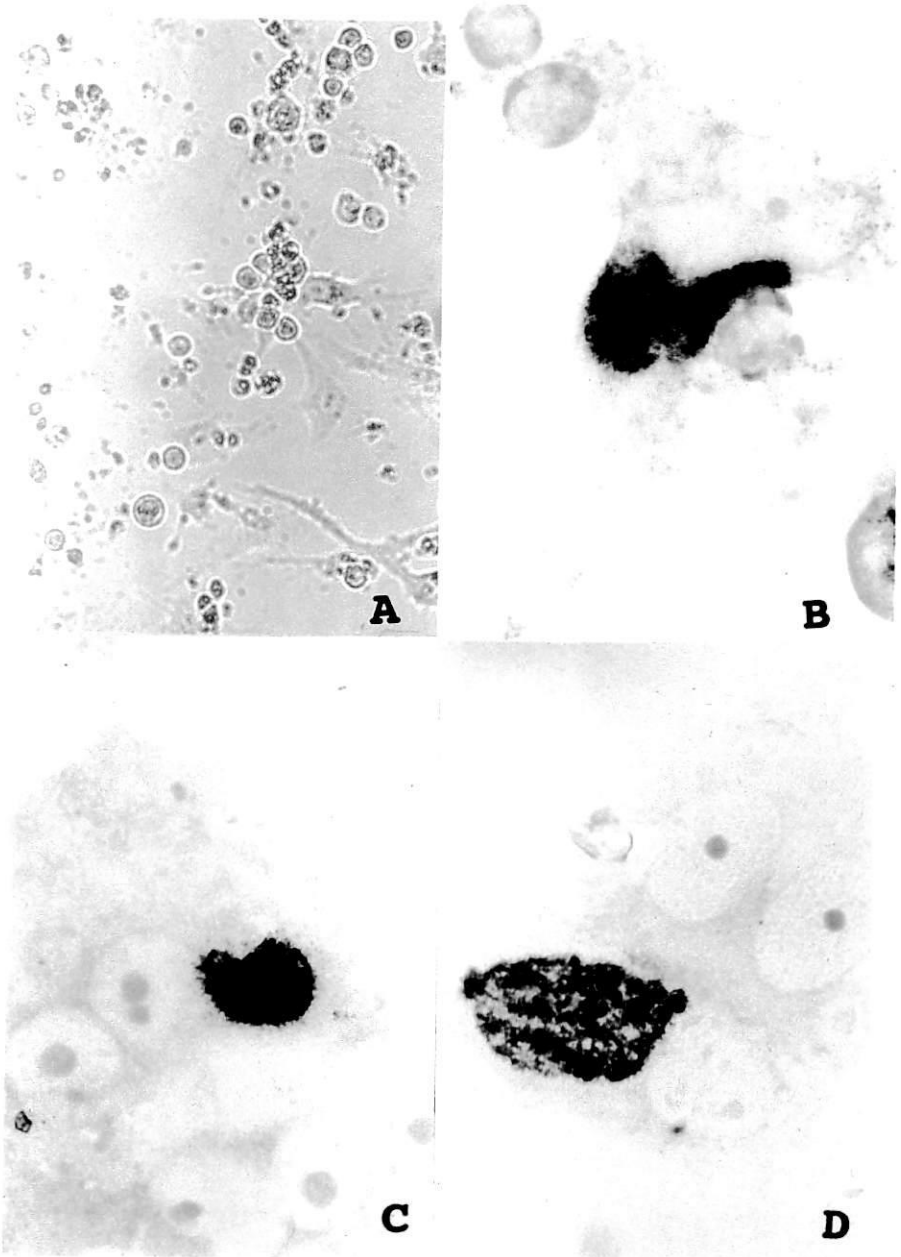

Figure 1 Cell fusion of cl.S61 cells to lymphoblastoid cells. Raji cells were cocultured with cl.S61 cells treated with IdUR for 2 h then were photographed under an inverted microscope (A). cl.S61 cells treated with IdUR were cocultured for 2 days with Raji (B), BJAB (C) and Jurkat (D) cells labeled with [^{3}H]-thymidine and labeled nuclei were detected by in situ autoradiography.(Panel A magnification, x100; all other magnifications, x400.)

cells, which lack virus receptors, did not form aggregations around cl.S61 cells, but Jurkat cell nuclei were still detected in multinucleated cl.S61 cells (Fig. 14), although the frequency was much lower than that of Raji and BJAB cells. These results indicate that cl.S61 cells treated with IdUR fused with not only B cell lines expressing EBV receptor/CR2 on their surfaces but also with cells devoid of it, although the virus receptor may function in the formation of aggregations around cl.S61 cells which enhance fusion efficiency.

It has been reported that EBV fused Raji cells by superinfection with strains of P3HR-1 and NPC-KT viruses (15,16,17), and that Raji cells superinfected with P3HR-1 virus fuse to virus receptor negative cells (16). However, viral factors reponsible for cell fusion appeared to be different between superinfected Raji and IdUR-treated cl.S61 cells, because fusion of Raji cells by superinfection with EBV was not inhibited by ACV (16), indicating that the fusion-inducing factor in superinfected Raji cells belongs to the early group of virus-specific protein. On the contrary,

syncytia formation of cl.S61 cells was inhibited by ACV and 2-deoxyglucose, indicating that viral glycoprotein belonging to the late group is responsible for cl.S61 cell fusion (12). The EBV late glycoprotein encoded by BZLF4 reading frame (gp110) has a sequence homology with herpes simplex virus 1 glycoprotein B, which is required for virus entry into cells and cell fusion (18) and is expected to play an important role in the fusion of cl.S61 cells. However, both IdUR-treated cl.S61 and superinfected Raji cells fused to cells lacking viral receptors, suggesting that although viral factors responsible for cell fusion in these cells differ, they share similar mechanisms of cell fusion. In cl.S61 cells, viral transcription factors and also viral genome may be transfered by cell fusion to neighboring cells, leading to the spread of the viral lytic cycle, and by the same mechanism, viral genome may be transfered to virus receptor negative cells.

In this communication, we have demonstrated that the EBV replicative cycle spreads via cell fusion in NPC-derived cl.S61 cells and that EBV

producing cells can fuse to both EBV receptor positive and negative cells, raising the possiblility of virus entry into EBV receptor negative cells by fusion.

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