Comparative Functional Analysis of the Various Lentivirus Long Terminal Repeats in Human Colon Carcinoma Cell Line (SW480 Cells) and Feline Renal Cell Line (CRFK Cells)

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ABSTRACT. Basal promoter activities of various lentiviral long terminal repeats (LTRs) in a human colon carcinoma cell line (SW480 cells) and a feline renal cell line (CRFK cells) were examined by the chloramphenicol acetyltransferase (CAT) assay using the LTR-CAT reporter plasmids. In SW480 cells, the basal promoter activities induced by LTRs of visna virus, caprine arthritis-encephalitis virus (CAEV), and simian immunodeficiency virus (SIV_{AGM}) were moderate, and those induced by LTRs of human immunodeficiency virus (HIV) type 1 (HIV-1) and HIV type 2 (HIV-2) were low. However, the activity induced by the LTR of feline immunodeficiency virus (FIV) was extremely low. In CRFK cells, the basal promoter activities induced by LTRs of visna virus, CAEV and SIV_{AGM} were relatively high, and those induced by LTRs of HIV-1, HIV-2 and FIV were moderate. From these data, although the structure of the LTR of FIV is reported to be similar to that of visna virus and CAEV, the function of the LTR of FIV is rather quite different from that of the LTR of these viruses.—**KEY WORDS:** CRFK cell, FIV, lentivirus, promoter activity, SW480 cell.

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Genus *lentivirus* members consist of human immunodeficiency virus (HIV) type 1 (HIV-1) and HIV type 2 (HIV-2), simian immunodeficiency viruses (SIVs), visna virus, equine infectious anemia virus, caprine arthritis-encephalitis virus (CAEV), feline immunodeficiency virus (FIV) and bovine immunodeficiency-like virus [21]. Genetic complexity is characteristic to all lentiviruses. In addition to the genes for virus structural and enzymatic proteins (Gag, Pol, and Env), which are essential for all retroviruses, the lentivirus genome contains several short open reading frames which encode a number of auxiliary proteins [3, 20]. Transcription of lentiviral mRNA from long terminal repeat (LTR) is regulated by the virally encoded auxiliary proteins such as Tat and Nef, and by cellular transcription factors which interact with the enhancer/promoter region located in the U3 region of the LTR.

In the LTRs of the respective lentiviruses, many binding sites for cellular transcription factors are present. NF κ B and SP1 binding sites are conserved among primate lentiviruses [4, 20], but not among FIV, visna virus and CAEV [9, 10, 15, 17, 20, 24, 30]. Instead, the latter viruses commonly possess putative binding sites for AP-1, AP-4 and C/EBP in their LTRs [9, 10, 15, 17, 20, 24, 30]. Of them, AP-1 and/or AP-4 binding sites were shown to be critical for efficient transcription of visna virus [10] and FIV [12, 19, 30]. From these observations, the structure and function of FIV LTR were suspected to be similar to

those of visna virus and CAEV [20].

Previously, we reported that although the FIV TM1 strain isolated in our laboratory can not infect either a feline renal cell line (CRFK cells) which is permissive for FIV Petaluma strain isolated in the United States, or a human colon carcinoma cell line (SW480 cells), the infectious molecular clone of FIV TM1 strain can replicate in the CRFK cells but not in the SW480 cells [18]. In addition, we found that the promoter activity of the FIV in the CRFK cells is rather high, but that in the SW480 cells is quite low [18]. Quite recently, we also reported that a viral determinant of infectivity of FIV for CRFK cells is located in the *env* region [13]. From these data, we concluded that, whereas the primary block to FIV infection of certain cells might occur at the cell surface, the FIV LTR may also participate in controlling virus replication, as an intracellular mechanism [18]. In this report, to determine whether the low promoter activity in the cells is specific for FIV or common to other viral promoters, especially LTRs of visna virus and CAEV, we examined and compared the promoter activities of LTRs of various lentiviruses including visna virus and CAEV in the SW480 and CRFK cells.

MATERIALS AND METHODS

Cells: SW480 (human colon carcinoma cell line [1]) and CRFK (feline renal cell line [2]) cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum as described previously [18].

DNA constructs: The chloramphenicol acetyltransferase (CAT) constructs under the control of the various viral promoters are shown in Fig. 1. They were made by placing virus LTRs or promoters in front of the CAT gene and SV40 poly A signal and small t intron. The expressions of

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Fig. 1. Constructions of the reporter plasmids of various viral promoters. Abbreviations: MCS, multi-cloning site; poly A & t, poly A signal and small t intron of SV40.

CAT by pTM1CAT, pVisLTRCAT, pCAEVLTRCAT, pH1CAT, pH2CAT, pSACAT, pRSpCAT Δ R Δ DA and pFLCAT are regulated by the LTRs of FIV [11, 17], visna virus [9, 10], CAEV [9], HIV-1 [1], HIV-2 [28], SIV_{AGM} [29], Rous sarcoma virus (RSV) [6, 25], and feline leukemia virus (FeLV) [16, 23], respectively. The CAT expressions of pCMVCAT and pSV₂CAT is directed by the promoter region of human cytomegalovirus (CMV) immediate early (IE) gene [23, 27] and the early promoter of simian virus 40 (SV40) [7], respectively. The pHdCAT [28] which is a promoter-less reporter plasmid was also used as a control.

Transfection: For transfection of plasmid DNA, cells were plated in six-well dishes one day before transfection. Two μ g of reporter plasmid was transfected by the calcium phosphate coprecipitation method [8, 31]. Four hr after transfection, the cells were washed with phosphate-buffered saline, shocked with glycerol, and then fed with fresh medium.

CAT assay: For the CAT assay, cells were harvested by scraping two days after transfection, suspended in 100 μ l of 0.1 M Tris hydrochloride (pH 7.8), and then lysed by four cycles of freezing and thawing. Cell lysates were clarified by centrifugation for 5 min at 4°C, and the resulting supernatants were treated at 65°C for 10 min to inactivate inhibitors of CAT. The denatured protein was removed by centrifugation for 1 min at 4°C, and the resulting supernatants were then assayed for CAT activity, which was carried out by the solvent partition method of Neumann *et al.* [22]. In brief, a 240 μ l reaction mixture containing 100 mM Tris hydrochloride (pH 7.8), 1.0 mM chloramphenicol, 3.7 KBq of [¹⁴C] acetyl coenzyme A (Du Pont, NEN), and 10 μ l (CRFK cells) or 30 μ l (SW480 cells) of cell extract was overlaid with 5 ml of scintillation fluid (Econofluor; Du Pont, NEN). Reactions were carried out at room temperature. Production of radioactively labeled acetyl-chloramphenicol was monitored by counting in a liquid scintillation counter, and then the CAT activity was expressed as the net dpm product formed per hr, and calculated by the formula as follows: (([CAT activity of each plasmid at 3 hr] - [CAT activity of pHdCAT at 3 hr]) - ([CAT activity of each plasmid at 1

hr] - [CAT activity of pHdCAT at 1 hr]))/2.

RESULTS AND DISCUSSION

Figure 2 shows the representative CAT assay data obtained in this study. The CAT activity induced by each reporter plasmid increased almost linearly until around 125,000 dpm. Therefore, the CAT activity of each reporter plasmid was calculated by the formula described in the section of Materials and Methods.

Since RSV LTR is known to have strong promoter activity in a variety of eukaryotic cells [6], the CAT activities of various reporter plasmids were represented as a percentage of those of pRSpCAT Δ R Δ DA (RSV LTR CAT plasmid) which showed high promoter activities in both cell lines (Fig. 3).

In SW480 cells (Fig. 3a), the basal promoter activities of the LTRs of visna virus, CAEV and SIV_{AGM}, and by human CMV IE promoter were moderate, and those of induced by LTRs of HIV-1, HIV-2 and FeLV, and by the SV40 early gene promoter were relatively low. However, the activity induced by LTR of FIV was extremely low in the cells as described previously [17].

In CRFK cells (Fig. 3b), the basal promoter activities induced by LTRs of visna virus, CAEV and SIV_{AGM}, and by human CMV IE promoter were relatively high, and those by LTRs of HIV-1, HIV-2, FIV and FeLV, and by SV40 early gene promoter were moderate.

In this study, we used each viral promoter from only one strain of the respective viruses and it might be possible that the promoter activities of respective viruses show strain-difference. Therefore, we could not exclude the possibility that each viral promoter activity in the cells is not representative. However, it was reported that the LTRs of several strains of SIV_{AGM} showed similar promoter activities in the SW480 cells [26], and that no significant difference was observed between the LTRs of FIV TM1 and Petaluma strains in CRFK cells and *Felis catus* whole foetus-4 cells (feline macrophage-like cell line) [13].

Phylogenetic analysis based on the highly conserved 90 amino acids from the reverse transcriptase domain of the *pol* gene revealed that FIV is closely related to visna virus and CAEV [5]. Further, transcriptional patterns and genome structures are similar among visna virus, CAEV and FIV [20]. In addition, the group of primate lentiviruses and of visna virus, CAEV and FIV, have similar structures of LTRs within the respective groups [20]. Therefore, it was expected that the LTRs in each group show similar promoter activities in both SW480 and CRFK cells. In this study, we found that LTRs of human lentiviruses (HIV-1 and HIV-2) showed similar promoter activities in both cell lines, and those of ruminant lentiviruses (visna virus and CAEV) did also. However, contrary to our expectation, the promoter activities of visna virus and CAEV were moderate in SW480 cells and high in CRFK cells, although the promoter activity of FIV was extremely low in SW480 cells, but high in CRFK cells.

Fig. 2. Basal promoter activities of various viral promoters in SW480 (a) and CRFK (b) cells. Two μg of each plasmid was transfected into the cells, and the CAT production in 30 μl (SW480 cells) and 10 μl (CRFK cells) out of 100 μl of each cell extract was measured by the CAT assay. These results are representative of three independent experiments. As the reporter LTR CAT constructs, pTM1CAT (FIV), pH1CAT (HIV-1), pH2CAT (HIV-2), pSACAT (SIV), pRSpCAT $\Delta\text{R}\Delta\text{DA}$ (RSV), pVisLTRCAT (Visna), pCAEVLTRCAT (CAEV), pFLCAT (FeLV), pCMVCAT (CMV), pHdCAT (control) and pSV₂CAT (SV40) were used.

Fig. 3. Basal promoter activities of various viral promoters in SW480 (a) and CRFK (b) cells expressed as relative CAT activities (%) in comparison with those of pRSpCAT $\Delta\text{R}\Delta\text{DA}$ (RSV) in respective cells. The averages and standard deviations from 3 independent experiments are shown.

Further, the LTR of SIV_{AGM}, belonging to the primate lentiviruses, showed rather higher promoter activities in both cell lines than those of HIV-1 and HIV-2 as described previously [18, 26]. From these data, we conclude that the quite low promoter activity in SW480 cells is specific for FIV. Moreover, from the measurements of the promoter

activities in both cell lines, it is considered that although the LTR of FIV is structurally similar to those of ruminant lentiviruses, the LTR is functionally different from those of ruminant lentiviruses as well as primate lentiviruses. Further, SIV_{AGM} seems to be similar to ruminant lentiviruses which shows relatively high promoter activities.

The reason for the extremely low promoter activity of the FIV LTR in the SW480 cells is unknown at present. However, it is possible that some suppressor factors are present in the cells, or some factors which are essential for the promoter activity are deficient in the cells. Using a 5' sequential deletion LTR mutant which was linked to the CAT gene and poly A signal in the CAT assay, we could not find any distinct suppressive element upstream from the promoter region of the LTR (data not shown). Further functional analyses of the FIV LTR are required to understand the mechanism of the low promoter activity in the SW480 cells. As several inhibitors of the promoter activity and replication of HIV-1 have been reported [14], the present study may be of use in the development of possible antiviral drugs which inhibit the promoter activity and replication of FIV.

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