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Androgen-independent proliferation of LNCaP prostate cancer cells infected by xenotropic murine leukemia virus-related virus

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ABSTRACT

Xenotropic murine leukemia virus-related virus (XMRV) is a novel gammaretrovirus that was originally isolated from human prostate cancer. It is now believed that XMRV is not the etiologic agent of prostate cancer. An analysis of murine leukemia virus (MLV) infection in various human cell lines revealed that prostate cancer cell lines are preferentially infected by XMRV, and this suggested that XMRV infection may confer some sort of growth advantage to prostate cancer cell lines. To examine this hypothesis, androgen-dependent LNCaP cells were infected with XMRV and tested for changes in certain cell growth properties. We found that XMRV-infected LNCaP cells can proliferate in the absence of the androgen dihydrotestosterone. Moreover, androgen receptor expression is significantly reduced in XMRV-infected LNCaP cells. Such alterations were not observed in uninfected and amphotropic MLV-infected LNCaP cells. This finding explains why prostate cancer cell lines are preferentially infected with XMRV.

1. Introduction

Xenotropic murine leukemia virus-related retrovirus (XMRV) is a novel human gammaretrovirus that was originally isolated from human prostate cancer tissues [1]. Although it is widely believed at present that XMRV is not the etiologic agent of prostate cancer, human prostate cell lines are frequently infected with XMRV [2].

It is known that some retroviruses play a critical role in leukemogenesis in various mammalian species including human [3,4]. The xenotropic MLV infection receptor (XPR1), which is also recognized by XMRV [5,6], varies among wild mice species as a mechanism of resistance to xenotropic virus infection [7,8]. The latter observation suggests that xenotropic viruses may be pathogenic in some species and implies that XMRV may affect growth of certain cell lineages.

Prostate cancer cell lines exhibit a propensity for infection by XMRV when compared to other types of human cancer cell lines [2,9]. It has been reported that amyloidogenic fragments originating from prostatic acid phosphatase greatly increase XMRV infections of primary prostatic epithelial and stromal cells [10]. In vivo infection of macaques with XMRV has confirmed that prostate tissue has a high affinity for XMRV, and the prostate tissues remain continuously infected even after 5 months, when XMRV was undetectable in blood [11]. Dihydrotestosterone (DHT) stimulates XMRV expression in cells expressing a functional androgen receptor (AR) [12,13]. These results suggest that XMRV infection specifically confers an advantage to prostate cancer cells.

In this study, we aimed to determine whether XMRV infection affects androgen-dependent growth of the LNCaP human prostate cancer cell line. Our results indicate that XMRV infection may provide an androgen-independent growth advantage to prostate cancer cells.

2. Materials and methods

2.1. Cells

PC-3 and LNCaP cells were obtained from ATCC. PC-3 cells were cultured in RPMI 1640 medium (Wako) supplemented with 8% (v/v) fetal bovine serum (FBS) (Biofuies), L-glutamine and penicillin–streptomycin (both from Sigma–Aldrich). LNCaP cells [14] were maintained in the same medium but additionally supplemented with 10 nM dihydrotestosterone (DHT) (Sigma–

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Aldrich). Rat F10, human HeLa, and human 293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 8% FBS and penicillin–streptomycin. All cell lines were grown in a tissue culture incubator at 37 °C with a 5% CO_2 atmosphere.

2.2. Retrovirus infection

The XMRV plasmid DNA was obtained from Dr. R.H. Silverman and Dr. B. Dong [1] through the AIDS Research and Reference Reagent Program (NIAID, NIH, USA) and was used for transfection of rat F10 cells. Culture supernatants of transfected F10 cells were used to inoculate target cells in presence of polybrene (4 μg/ml) (Sigma). Inocula containing MLV were from culture supernatants of amphotropic MLV-producing cells, obtained from Dr. Y. Iwatani. Infected LNCaP cells were maintained in presence of DHT. In tests of androgen responses, target cells were cultured in various combinations of DHT (10 nM) and bicalutamide (10 μM).

2.3. Cell counts and viability

The cells to be counted were collected and stained with trypan blue. Numbers of unstained (viable) cells were counted using a counting chamber under a microscope to estimate cell viability.

2.4. Western blot analysis

Cell lysates were subjected to electrophoretic separation in SDS-containing polyacrylamide gels (BioRad), after which proteins were transferred onto a PVDF membrane. The membrane was first treated with the primary antibodies: mouse anti-β-actin (Santa Cruz Biotechnology), goat anti-dynamin (Santa Cruz Biotechnology), rabbit anti-human AR (Santa Cruz Biotechnology), goat anti-MLV p30 Gag (ViroMed), or goat anti-MLV SU (ViroMed) antibody. Following these procedures, the membrane was treated with secondary horse radish peroxidase (HRP)-conjugated anti-mouse IgG antibody, or HRP-conjugated protein G (Bio-Rad). Secondary antibody- or protein G-bound polypeptides were detected by ECL Western Blotting Detection Reagents (GE healthcare).

2.5. Semi-quantitative RT-PCR

Total RNA and genomic DNA samples were isolated by standard protocols. Fist-strand cDNA was synthesized using reverse transcriptase (TaKaRa) from the total RNA (500 ng). Semiquantitative PCR was performed to detect XMRV env, AR, and GAPDH sequences. Nucleotide sequences of the PCR primers for the XMRV *env* sequences were 5′-GACTTGTGTGATTTAGTTGGAGAC-3′ and 5′-CCCCGGTGTGGCACC-3′; for AR, 5′-AGCCCCACTGAGACAACC-3′ and 5′-ATCAGGGGCGAAGTAGAGCAT-3′; and for GAPDH, 5′-AGG TXGGAGTXAAXGGATTTGGT-3′ and 5′-GTGGGCCATGAGGATCCAC-CAC-3′. These primers were synthesized by Genenet Inc.

2.6. Statistical analysis

Differences between two sets of data were determined by Student's *t*-test, and these differences were considered significant when $P < 0.05$.

3. Results

3.1. XMRV infection converts LNCaP cells to an androgen-independent phenotype

To analyze the effect of XMRV infection on androgen-dependent growth of LNCaP cells, the proliferation of XMRV-infected and -uninfected LNCaP cells was compared. As it has been reported that XMRV can replicate in rat cells but not in human 293T cells [12,13,], virus was first rescued by transfection of an XMRV expression plasmid [1] in rat F10 cells. Undiluted culture supernatant from these cells was then added to LNCaP cells with polybrene and cultured for 24 h. The XMRV-infected and -uninfected cells were maintained in the presence of 10 nM DHT for more than 3 months. Uninfected LNCaP cells did not grow in the absence of DHT (Fig. 1A) but did in the presence of DHT (Fig. 1B), indicating a strong androgen-dependent growth requirement, as reported [14]. As a control for specific AR effects, DHT-induced growth of uninfected LNCaP cells was shown to be abrogated by the antagonist bicalutamide, an androgen blocker (Fig. 1C). Bicalutamide (10 μM) alone had no effect on the growth of either infected or uninfected LNCaP cells (Fig. 1D). LNCaP cells chronically infected with XMRV grew even in the absence of DHT (Fig. 1A), and bicalutamide did not suppress growth of XMRV-infected LNCaP cells (Fig. 1C and D). In the presence of DHT, the number of XMRV-infected LNCaP cells was greater than control uninfected cells after 3 days in culture (Fig. 1B). Three independent XMRV-infected LNCaP cell pools were constructed, and all of them could grow in the absence of DHT. When uninfected LNCaP cells were maintained in the presence of DHT, the cells did not gain androgen-independent growth property during this study. These results indicate that XMRV infection converts LNCaP cell growth from androgen dependence to independence.

On the other hand, LNCaP cells chronically infected with amphotropic MLV did not efficiently proliferate even in the presence of DHT (Fig. 1A–D), suggesting that the amphotropic MLV infection is cytotoxic for LNCaP cells.

To determine the time course of the conversion of XMRV-infected LNCaP cells to androgen independence, growth kinetics were analyzed after XMRV infection (from 1 to 2 months, 2 to 3 months, and >3 months). Cultures initially contained 5×10^3 cells and were counted again after 6 days, because differences between the uninfected and XMRV-infected LNCaP cells in androgen dependence were apparent 6 days after the culture was started (Fig. 1A–D). DHT dependence of LNCaP cell growth was reduced by XMRV infection, but DHT still activated cell proliferation 1–2 months after XMRV infection (Fig. 1E). Cell numbers of the infected LNCaP cells in the absence of DHT were comparable to those in its presence 2–3 months after infection. These results suggest that the complete conversion of LNCaP cells to androgen independence takes more than 2 months. The XMRV infection did not increase cell numbers in the absence of DHT 1–3 months after the XMRV inoculation, but cell increases were observed longer than 3 months after inoculation, showing that the activation of LNCaP cell growth by the XMRV infection requires at least 3 months.

Uninfected PC-3 cells, whose growth is androgen-independent [15], grew as efficiently as XMRV-infected PC-3 cells in the absence or presence of DHT (data not shown). These results indicate that XMRV infection did not affect growth of androgen-independent PC-3 cells.

3.2. XMRV infection inhibits androgen receptor expression in LNCaP cells

Because androgen agonistic (DHT) and antagonistic (bicalutamide) effects are mediated through androgen receptor (AR), we analyzed the effects of XMRV infection on its expression in LNCaP cells. As demonstrated by western blot analysis, the expression of AR protein gradually decreased after XMRV infection of LNCaP cells (Fig. 2A). Expression was significantly decreased but still detectable 2–3 months after infection, but by >3 months no expression was observed. Therefore, the reduction of AR