Research Article

Bovine Leukemia Virus Non-Productive Infection of Human Mammary Epithelial Cells (MCF10A)

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ABSTRACT
Bovine leukemia virus (BLV) is a retrovirus that causes lymphosarcoma in cattle. Some researchers suggest that BLV could be related to breast cancer development, however, evidence that the virus can infect the human counterpart is lacking. For that reason, the objective of this study was to infect in vitro a human mammary epithelial cell line (MCF10A) with BLV. The results suggest that the infection is non-productive, since only a fragment of the viral gene pol was detected in the cellular DNA. These results are consistent with previous studies, where fragments of different BLV genes were found in human mammary tissue. Future studies should investigate whether this non-productive infection can be associated with human breast cancer.

Impacts
Bovine leukemia virus can infect a human mammary epithelial cell line and is integrated as a provirus. BLV infection on human mammary epithelial cell line (MCF10A) is not productive. BLV non-productive infection of the human mammary epithelial cells can be involved in breast cancer development.

Introduction
Bovine leukemia virus (BLV) is a δ-retrovirus that affects cattle causing a lymphoproliferative disease. Its main target cell is the B lymphocyte but, naturally and experimentally, this virus can infect other cells such as neutrophils, epithelial and brain cells [1-4]. Since the detection of BLV in bovine epithelial mammary cells, researchers have considered the possibility of this virus infecting human epithelial mammary cells [2, 5, 6]. Many facts support this idea. First, BLV belongs to the same viral family that the human T lymphotropic virus (HTLV). This is a carcinogenic virus in humans and shares structural and functional homology with BLV [7]. Although the BLV receptor in cattle is not yet elucidated, it is believed that it is a protein from the adaptor-related protein complex 3 (AP-3) [8]. This protein is also present in humans, who have four different AP complex. The fact that the putative receptor of the virus has 88% homology with the human counterpart and that the putative binding site for the viral protein gp51 is completely homologous in both species support the possibility of BLV infecting humans [9].

Virus have been associated with the development of different tumors in humans. It is believed that around 12% of the cancer cases are from viral origin [10]. The possible role of BLV in the development of human breast cancer has been investigated in the past years with controversial results [11-15]. Numerous studies have detected fragments of viral genes and proteins in human breast samples using different techniques such as...
in situ PCR, immunohistochemistry, and conventional PCR [13-18]. Most of these studies suggest that the virus might be implicated in the onset or development of human breast cancer. However, to our knowledge, no study unequivocally demonstrates whether the virus can infect human breast cells. Confirming that the virus can infect human mammary cells is the first step to analysing the possible relationship between the virus and the development of human breast cancer. Thus, the aim of this study was to infect in vitro a human mammary epithelial cell line (MCF10A) and analyse its effects.

Materials and Methods

BLV was obtained from BLV-infected adult cows in the Manhattan area (Kansas, USA). From each animal, 20 ml of heparinized blood (5 U ml⁻¹) were obtained by jugular venipuncture. Peripheral blood mononuclear cells (PBMCs) were isolated using SepMate™ (STEMCELL Technologies Inc, Canada) and Ficoll-Paque™ PREMIUM (GE Healthcare) following the manufacturer’s instructions. PBMCs were cultivated in RPMI supplemented with 10 % FBS by incubating at 37°C with 5% CO₂ until use.

For MCF10A infection, forty thousand cells were seeded in each well of a six well plate and cultured until they reached 90% confluence. The cells were co-cultured with 1 x 10⁴ PBMCs from the infected animals for 24 h. After that time, cells were passed three times (to passage 3 post infection; 3 ppi) and started to be analysed. The experiment was conducted in triplicate using PBMCs from three different animals. The infected cell line was designated MCF10A BLV. DNA was extracted from cell pellets using Qiagen columns (QIAamp DNA Mini Kit, Germantown, MD, USA) according to the manufacturer’s protocol. DNA concentration and purity were determined by absorbance at 260 nm and 260/280 coefficient, respectively, in a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Purified DNAs were stored at -20°C until use.

Obtained DNA was used as a template to amplify the viral gene pol, as well as the human and bovine GAPDH by conventional PCR. All three PCR reactions were performed in a final volume of 25 µl containing 12.5 µl of GoTaq MasterMix (Promega®), 0.4 mM primers and 1 µl of the template. The primers and PCR program used for pol and bGAPDH were described previously [16, 19]. For bGAPDH amplification the primers used were F: 5’ TGGTGTGGATCTGCCGTGC 3’ and R: 5’ TGAAGGGCTGTTTACCGAGC 3’ and the amplification was performed in MCF10A BLV genomic DNA. DNA was extracted, and a fragment of the viral gene detected in vitro was amplified in MCF10A BLV genomic DNA. The statistical analysis was performed in R using ANOVA test.

Results and Discussion

Our results show that human mammary cell line MCF10A can be infected with BLV in vitro. The new cell line called MCF10A BLV resulted from the infection of MCF10A with PBMC from highly BLV infected cows. The analysis of this new cell line began at passage 3 ppi where genomic DNA was extracted, and a fragment of the viral gene pol was detected by conventional PCR. DNA obtained from MCF10A BLV and the positive control amplified a unique fragment of the expected size of 184 pb (Figure 1). Moreover, when using MCF10A BLV DNA as a template, only human and not bovine GAPDH could be amplified, dismissing the possibility that the fragment of the pol gene detected in MCF10A BLV cells might come from remaining bovine PBMCs used for infection.
The 24 kDa viral protein was not detected in cells supernatant which suggest that the new cell line does not release viral particles into the cell culture supernatant. Considering the possibility that the viral protein p24 was synthesized but unable to be released, cell pellets were lysed for analysis. The protein was not detected by Western blot in the cell lysates nor was it found in MCF10A-BLV supernatant (Figure 2a). The absence of this viral protein in cell culture supernatants was confirmed up to 30 ppi.

To confirm that the cells were not releasing virus, an RT-PCR assay was carried out to detect RNA from the viral gene pol in cell culture supernatants and pellets. No amplification was observed in the supernatants (data not shown). To analyse the presence of the viral gene in cell pellets, a kit that extracted both viral DNA and RNA was used (MagMAX™-96 Viral RNA Isolation kit). When these nucleic acids were analysed by RT-PCR, the fragment of a viral gene pol was amplified. However, when a DNase treatment was carried out before the RT-PCR, amplification of the pol fragment was no longer detectable (Figure 2b). Cell viability analysed by MTT showed no difference between BLV infected and uninfected MCF10A (p = 0.2857) for at least 33 ppi. It is known that non-productive infections could play a role in tumor development [20]. Although the virus cannot generate new infective viral particles, it can produce proteins that interfere with the normal cell cycle.

Our results show that BLV infected human epithelial breast cells have no difference in cell viability compared to the uninfected cells when measured by MTT. The reason for this lack of effect on the cell viability could be that the cells were not infected for enough time to suffer significant changes. Previous studies that analysed the presence of the virus in human mammary tissue found that the viral DNA fragments were present in healthy individuals up to 10 years before the development of breast cancer [14]. This suggests that if BLV does play a role in breast cancer tumorigenesis, it likely progresses very slowly and the effect of the virus on cell viability will not be evident for a long time. Moreover, in most virus-related cancer, the presence of the virus is not the only condition necessary to develop the tumor. Other factors such as immunosuppression, chronic inflammation, and spontaneous mutations contribute to the outcome. This could also explain why we did not find any difference in cell viability in vitro.


In summary, we have infected a human mammary cell line with BLV in vitro. The new cell line does not release viral particles into the cell culture supernatant. Nevertheless, viral DNA was detected in the mammary epithelial cell genome. Previous studies indicate that the difference between BLV productive and non-productive infection is the efficiency in the reverse transcription [21]. In this case, we demonstrated that the virus is integrated, which indicates that the reverse transcription is not the critical step for BLV replication in this cell line. Moreover, it is well known that retrovirus expression can be repressed by epigenetic mechanisms both in natural and interspecies infections [22]. This could also explain the fact that no viral proteins were detected in MCF10A BLV cells supernatants.

More research needs to be done to elucidate if BLV plays a role in human breast cancer. The fact that we were able to detect a fragment of the viral gene pol in the MCF10A DNA is consistent with previous studies that detected other gene fragments by PCR and in situ PCR. Although the infection in MCF10A was not productive, we cannot disregard the possibility that this virus can play a role in human breast cancer.

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Conflicts of Interest

None.

REFERENCES


3. Lucía Martínez Cuesta, Maria Victoria Nieto Farias, Pamela Anahi Lendez, Lucas Barone, Sandra Elizabeth Pérez et al. (2018) Stable infection of a bovine mammary epithelial cell line (MAC-T) with bovine leukemia virus (BLV). *Virus Res* 256: 11-16. [Crossref]


5. James S Lawson, Brian Salmons, Wendy K Glenn (2018) Oncogenic Viruses and Breast Cancer: Mouse Mammary Tumor Virus (MMTV), Bovine Leukemia Virus (BLV), Human Papilloma Virus (HPV), and Epstein-Barr Virus (EBV). *Front Oncol* 8: 1. [Crossref]


7. Sabrina M Rodríguez, Arnaud Florins, Nicolas Gillet, Alix de Bregnac, Maria Teresa Sánchez Alcaraz et al. (2011) Preventive and therapeutic strategies for bovine leukemia virus: Lessons for HTLV. *Viruses* 3: 1210-1248. [Crossref]


11. Nicolas A Gillet, Luc Willems (2016) Whole genome sequencing of 51 breast cancers reveals that tumors are devoid of bovine leukemia virus DNA. *Retrovirology* 13: 75. [Crossref]


