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Short Communication

Heme oxygenase-1-mediated host cell response inhibits the susceptibility of prostate cancer cells to retroviral infection and retards their proliferation

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ABSTRACT

Xenotropic murine leukemia virus-related virus (XMRV) resembles endogenous murine leukemia virus and was used in this study as a model for a new retrovirus infecting human cells. We demonstrate that induction of an HO-1-mediated host cell response inhibited the susceptibility of LNCaP prostate cancer cells to XMRV infection and efficiently retarded the growth of these prostate cancer cells. Our studies delineate a role of HO-1 in the host defense against retroviral infections and may provide novel therapeutic strategies for the treatment of HO-1-sensitive prostate cancer.

KEYWORDS: XMRV infection, heme oxygenase-1, gene expression, host factors

Despite previous reports suggesting XMRV as a causative agent of prostate cancer and chronic fatigue syndrome (CFS), with reported presence of the virus in the blood of CFS patients [1], the relevance and significance of this retrovirus in these disease conditions remain unconfirmed [2, 3]. Nonetheless, XMRV is a retrovirus that can infect a variety of human cells, including prostate cancer cells [4, 5]. Previously, we have shown that induction of HO-1, an endogenous host cell enzyme, inhibits HIV-1 infection *in vitro* and *in vivo* [6, 7]. Whether HO-1 can protect against other viral infections is not known. In this study, LNCaP human prostate cancer cells and XMRV were used as a model system to examine HO-1-mediated host defense against retrovirus infection and to determine its inhibitory role in tumorigenesis.

Treatment of LNCaP cells with hemin induced HO-1 expression in a dose-dependent manner (Fig. 1A). To examine the role of HO-1 in modifying XMRV infection, LNCaP cells were infected with XMRV in the absence or presence of 100 μM hemin for two hours, washed and replenished with fresh media with and without hemin for four days. Total RNA was isolated and examined for XMRV expression by real-time quantitative PCR using previously described reaction conditions [3]. The cycle threshold values (Ct) were normalized against human glyceraldehyde-3-phosphate dehydrogenase

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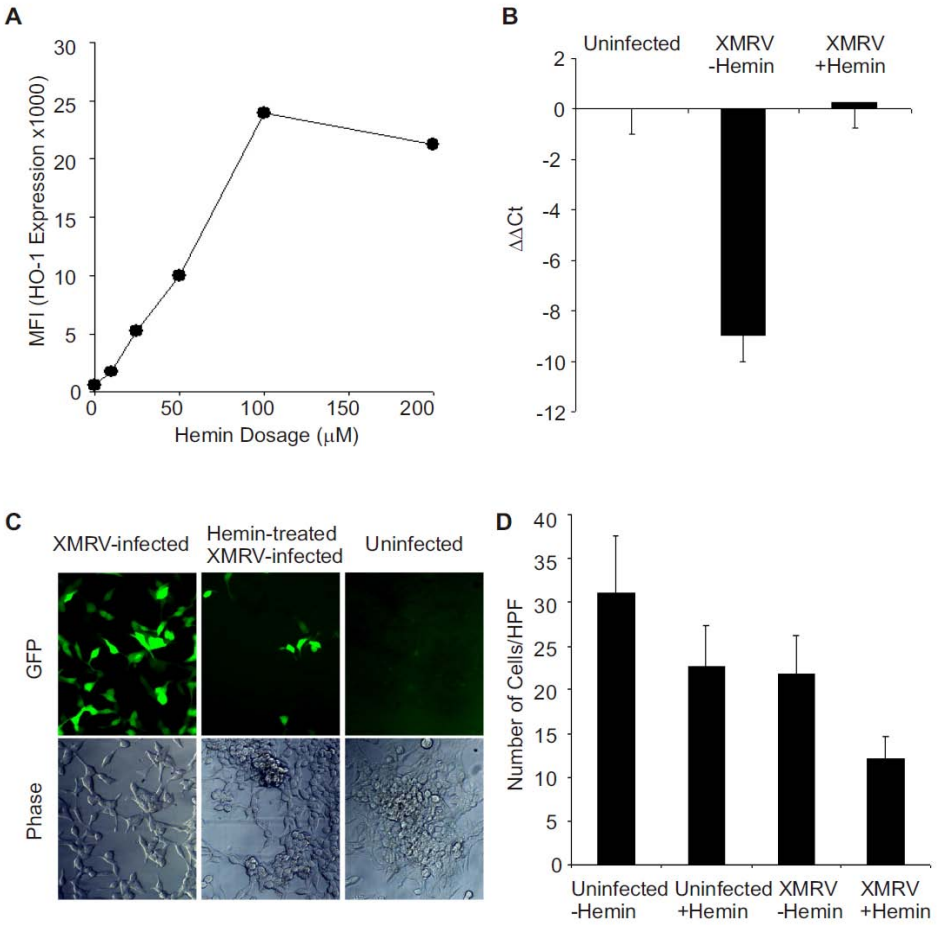

Fig. 1. HO-1 induction inhibits XMRV infection of LNCaP cells. A. LNCaP cells were treated for 24 h at 37°C with hemin at the indicated concentrations. After incubation, cells were washed with phosphate buffer saline, pH 7.2 (PBS), permeabilized using 0.05% saponin in PBS, stained using fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against HO-1, and analyzed for HO-1 expression by flow cytometry; **B.** LNCaP cells were infected with XMRV (10^6 copies/mL), washed with warm medium, and cultured for 4 days in the absence or presence of 200 μM hemin. Viral RNA levels were determined by quantitative real-time RT-PCR, and expressed as ΔΔCt values ((Target Ct value - GAPDH target Ct value) - (control Ct value - GAPDH control Ct value)). **C.** LNCaP cells infected with GFP-tagged XMRV in the absence or presence of 200 μM hemin were cultured for 7 days in the absence and presence of hemin and examined under a fluorescence microscope to visualize GFP-XMRV expression; **D.** After 7 days in culture, cells were detached using 0.25% trypsin, counted microscopically, and expressed as the number of cells/high power field.

(GAPDH) and expressed as ΔΔCt values. As shown in Fig. 1B, hemin treatment significantly inhibited XMRV infection of LNCaP cells. Consistent with these results, infection of HO-1-induced LNCaP cells with GFP-tagged-XMRV revealed a substantially reduced level of the virus in fewer cells (~10% of the cell population; Fig. 1C). In addition, HO-1 induction significantly retarded the growth of both uninfected and XMRV-infected LNCaP cells (Fig. 1D).

Most prostate cancer cells express surface receptors for calcitonin (CT), a hormone produced primarily by the parafollicular cells of the thyroid. Exogenous CT promotes cell proliferation, invasion, and tumorigenesis [8]. To examine the effect of CT on XMRV infection, uninfected and XMRV-infected LNCaP cells were cultured for 4 days in the absence and presence of 100 nM CT, and examined for the viral gene expression and cell growth. Although CT treatment slightly

altered viral RNA expression in the XMRV-infected cells (Fig. 2A), it stimulated the growth of both uninfected and XMRV-infected LNCaP by about two-fold after 7 days in culture (Fig. 2B). While no significant difference in CT-induced cell growth was seen between the uninfected and XMRV-infected cells, hemin treatment markedly arrested the CT-stimulated growth of LNCaP cells (Fig. 2C). Whole genome expression analysis of the reverse transcribed RNA revealed that host gene expression was largely unaffected by XMRV infection, in contrast to a recently published report [9]. Nonetheless, hemin activation of XMRV-infected LNCaP cells significantly induced HO-1 and HO-1-dependent host gene expression (Fig. 3A). The regulation of HO-1, ferritin heavy chain1 (FTH1) and transferring receptor (TRFC) was confirmed by real-time PCR using specific primers for these genes (Fig. 3B).

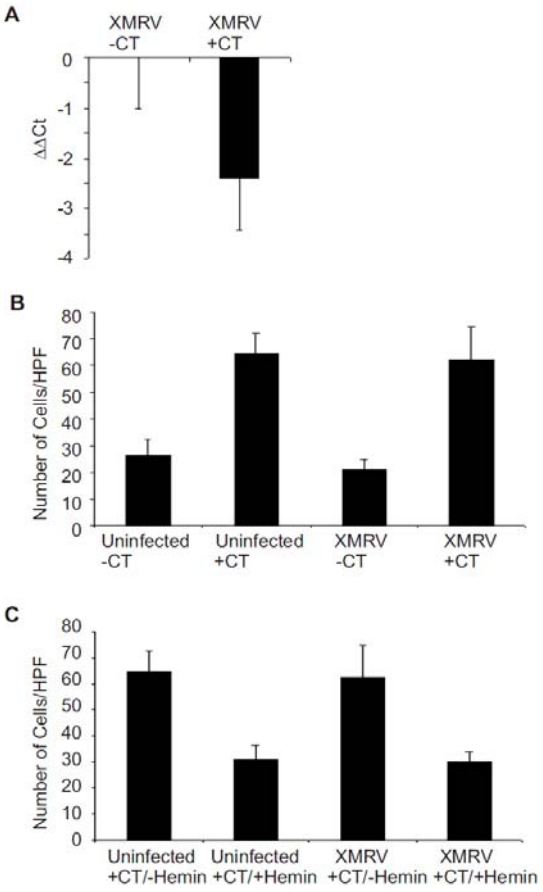

Fig. 2. A. Effect of calcitonin (CT) on XMRV infection of LNCaP cells. XMRV-infected cells were cultured for 4 days in the absence or presence of 100 nM CT, and examined for virus infection by RT-PCR as described in Fig. 1. **B. Effect of CT on the growth of uninfected and XMRV-infected LNCaP cells.** Uninfected and XMRV-infected LNCaP cells were cultured in the absence or presence of 100 nM CT. Culture media were replenished with fresh media (with or without CT) every other day. On day 7, cells were detached with 0.25% trypsin and counted microscopically. **C. Effect of hemin on the growth of uninfected and XMRV-infected LNCaP cells cultured in the presence of CT.** Uninfected and XMRV-infected LNCaP cells were cultured in the presence of 100 nM CT with or without 100 μM hemin. Culture media were replenished with fresh media (with or without CT) every other day. On day 7, cells were detached with 0.25% trypsin, counted microscopically, and expressed as the number of cells/high power field.

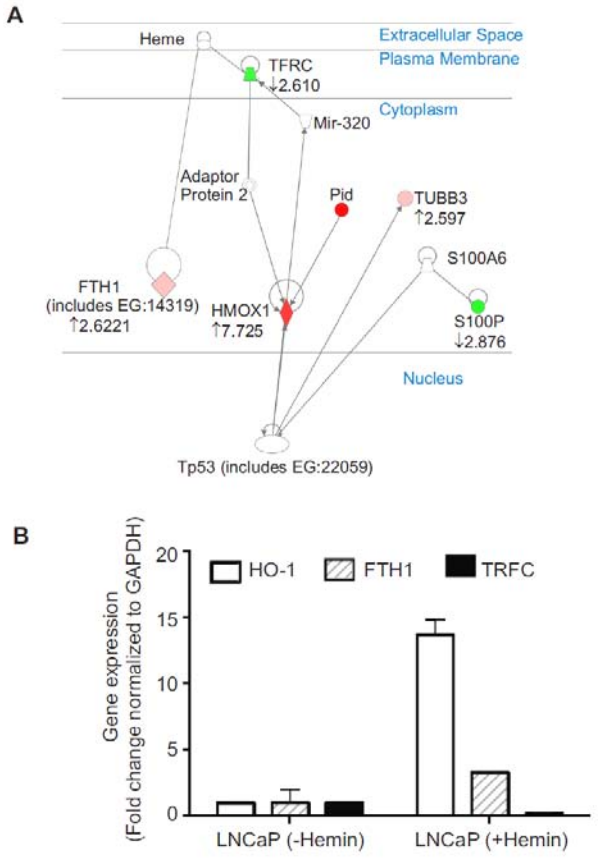

Fig. 3. A. Ingenuity Pathway Analysis of genes modulated by hemin treatment of XMRV-infected LNCaP cells. Genes up-regulated are red or pink colored and genes down-regulated are green colored. **B**. Real-time PCR of HO-1, FTH1, TRFC and GAPDH using specific primers for these genes. RNA was extracted from uninfected, XMRV-infected, and hemin-treated XMRV-infected LNCaP cells, reverse transcribed, and analyzed using a Roche 4800 real-time PCR system.

These findings indicate a role for HO-1 against XMRV infection of prostate cancer cells, and are consistent with the previously described HO-1-mediated inhibition of HIV-1 infection [5, 6]. Hemin, used as a potent HO-1 inducer in this and the previous study, is the active component of an FDA-approved biologic therapeutic for the treatment of acute porphyria. Since the 1970s, several formulations of hemin have been used successfully to treat various medical conditions [10, 11]. Therefore, subject to further studies, hemin induction of HO-1 could potentially provide a new therapeutic strategy for the treatment of HO-1-sensitive prostate cancer.

In summary, HO-1 induction by its physiological substrate hemin markedly inhibited XMRV infection of LNCaP cells *in vitro*. HO-1 induction markedly retarded the proliferation of both uninfected and XMRV-infected LNCaP cells, including those with calcitonin-stimulated growth. These results indicate that HO-1 induction may provide a new therapeutic strategy for the treatment of retroviral infections and HO-1-sensitive prostate cancer.

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