

## PREVALENCE AND DISTRIBUTION OF XMRV IN PROSTATE CANCERS AND OTHER HUMAN TISSUES

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Xenotropic Murine Leukemia Virus–like Retrovirus (XMRV) was recently discovered in human prostate cancers [1]. We generated a full-length infectious clone of XMRV using two overlapping cDNAs of the virus that were derived from human prostate cancer tissue [1]. Introducing our proviral DNA into 293T cells resulted in release of viral particles as seen by reverse transcriptase activity in the supernatants. LNCaP cells (prostate cancer cell line) were inoculated with this culture supernatant. Particles released from these infected cells were characterized by Western blot analysis using antibodies specific to XMRV. The pattern of viral protein bands resembled that of other xenotropic murine retroviruses. Particles of ~120 nm diameter and morphology typical for type-C retroviruses were seen by transmission electron microscopy [2].

XMRV was first detected in prostate cancer tissues from men homozygous for a reduced activity variant of an antiviral gene, *RNASEL*. Using a qPCR allelic discrimination assay we performed *RNASEL* genotyping of 233 cases with prostate cancer and 101 cases with benign prostatic hyperplasia. In contrast to initial reports [1], we found no association of the *RNASEL* variant genotype with either the presence of prostate cancer or the presence of XMRV. This finding increases the population at risk for XMRV infection from only those homozygous for the *RNASEL* variant (~10% of the population) to all individuals.

To study the prevalence and distribution of XMRV in human prostate tissues we developed a sensitive qPCR assay to detect XMRV proviral DNA, and rabbit antisera against whole XMRV. For primer and probe design we selected a region in the proviral DNA that would allow for efficient detection of XMRV without interference from related murine or other endogenous retroviral sequences. Genomic DNA was extracted from a total of 233 banked frozen or formalin-fixed paraffin-embedded prostate cancer tissues. Our qPCR assays revealed the presence of XMRV in 6% of frozen prostate cancer tissues. We also wished to see the distribution and expression of XMRV proteins within these prostate cancers. Using antisera generated by injecting rabbits with inactivated, detergent-lysed XMRV, we developed a sensitive immunohistochemical staining protocol. We found 23% of the 233 prostate cancers to stain positive for XMRV. In contrast to the initial report that found XMRV in rare benign stromal cells adjacent to malignant epithelium [1], our protocols revealed staining of malignant prostatic epithelial cells. Only in very rare cases was stromal staining seen, either with or without concomitant staining of malignant epithelium. The presence of virus in malignant cells invokes classic pathways for retroviral pathogenesis, i.e. inactivation of a tumor suppressor or activation of an oncogene by retroviral integration, as possible mechanisms of tumorigenesis.

Does XMRV infect human tissues other than the prostate? We examined all tissues collected from a series of autopsies performed on men with prostate cancer, and on men without prostate cancer. We will describe the distribution of XMRV in an individual with prostate cancer, and in various tissues examined from 72 consecutive autopsies performed on adult males who did not have prostate cancer.

1. Urisman, A., et al., Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q *RNASEL* variant. *PLoS Pathog*, 2006. 2(3): p. e25.
2. Schlaberg R., Choe D. J., Brown K. R., Thaker H. M., and I. R. Singh (2009) XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. *Proc. Natl. Acad. Sci. USA*, 106:16351-16356.