GEORGE MASON UNIVERSITY
COLLEGE OF SCIENCE
BIOLOGY DEPARTMENT SEMINAR
Spring 2016

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"Using a quasivirus-based Human Papillomavirus genome delivery system to study the modulation of early stage infectivity by host factors"

Human Papilloma Viruses (HPVs) are a family of highly ubiquitous small, double stranded DNA viruses, comprised of (low risk) benign and high risk (oncogenic tended) HPV strains. The HPV life cycle can be summarized in 3 stages: (1) initial infection and establishment, where it begins a short term replication and gene expression program to initiate the infection (2) a maintenance phase where it is maintained as a low copy episome in actively dividing basal keratinocytes and (3) a vegetative phase where the late stage of its life cycle coordinates with the differentiation program of its host keratinocytes to maximally replicate its genome and produce viral progeny. The chromatin-binding and HPV E2 interacting protein, BRD4, has been implicated in E2-mediated repression of alpha HPV early gene expression, is associated with host chromatin in complex with E2 and the viral genome, and colocalizes to very active sites of HPV DNA replication in concert with DNA damage response factors. However, the precise role of Brd4 in modulating viral transcription and replication is unclear, in part because viral genomes encoding E2 proteins that are unable to bind Brd4 are largely functional, when transfected into keratinocytes. In normal cellular gene expression, BRD4 is largely believed to be a transcriptional activator. By binding to acetylated lysine residues on chromatin, BRD4 is able to interact with positive transcriptional regulators and help initiate transcription. Since transfection of viral genomes or induction of late stage genome amplification in cell lines present situations of high genome copy number and do not reveal a direct biological role for BRD4, we've utilized a HPV18 virus-based genome delivery system to assess initial infectivity of primary keratinocytes at early stages when low levels of de novo-introduced viral genome would warrant optimal efficiency of viral-mediated processes. We have produced recombinant HPV18 genome-containing pseudoviruses (quasiviruses) in Human Embryonic Kidney (HEK) 293TT packaging cells by co-expressing HPV18 genome with the L1 and L2 capsid proteins. Moreover, heterologous co-expression of the HPV18 E1 and E2 replication proteins was sufficient to induce replication and enhance encapsidation of HPV18 genomes in 293TT cells. Upon infection of primary human keratinocytes with an HPV18 quasivirus, we show that BRD4 activates viral transcription and replication and that transcriptional activation is dependent on the functional interaction between BRD4 and the HPV18 E2 protein.

TUESDAY March 1, 2016
3:00-4:15 PM
Fairfax Campus: Innovation Hall Room 334
Science & Technology Campus: OCC 221