Beta-HPV E6 Contributes To Skin Cancer by Hindering DNA Repair

June 15, 2015

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High-risk alpha human papilloma viruses are well known for their role in cervical and oropharyngeal cancer. However, there are more than 100 members of this family of viruses that are human pathogens and additionally, some members of the beta-HPV group have been found to be associated with non-melanoma skin cancer (NMSC). While both alpha and beta HPVs are linked to cancer, their mechanisms of action appear to be markedly different. For instance, the E6 and E7 proteins from alpha-HPVs promote degradation of tumor suppressors, p53 and RB while similar proteins in beta-HPVs do not. In addition, NMSC linked to beta-HPVs does not require sustained expression of viral proteins. How, then, do beta-HPVs contribute to NMSC? Previous work in Dr. Denise Galloway's Laboratory (Human Biology Division) shed some light on this question by demonstrating that E6 proteins from beta-HPV 5 and 8 hindered the repair of UV damage through binding and destabilization of p300, an essential protein in the DNA damage response (Wallace et al., 2012). "When I joined Denise's lab, we were looking for evidence to support our theory that certain HPVs can make exposure to UV light more mutagenic and as a result more carcinogenic. In previous work, we showed that these same viral proteins impeded the repair of UV damage, increasing the likelihood that a UV lesion would become a more deleterious double strand break," said Dr. Nicholas Wallace.

A new Fred Hutch study by the Galloway lab, also led by postdoctoral fellow Dr. Nicholas Wallace and published in the journal *PLOS Pathogens*, tested the hypothesis that E6-mediated degradation of p300 leads to reduced expression of BRCA1, a protein famous for its role in inherited breast cancer but also known to be essential for homology-directed repair (HDR) of DNA double strand breaks (DSBs). As a first step to test this hypothesis, the authors induced DSBs with ionizing radiation in primary human cells that expressed E6 from beta-HPV 5 or 8. Compared to control cells, E6-expressing cells exhibited increased persistence of DSB markers (pH2AX and 53BP1). In contrast, a mutant E6 that can no longer bind p300 did not significantly alter DSB repair, suggesting that interaction with p300 was required for this effect. Because p300 was previously shown to be essential for BRCA1 expression, the researchers monitored the levels of BRCA1, as well as that of other HDR proteins, upon expression of beta-HPV E6. Immunoblotting analysis revealed a decrease in levels of both BRCA1 and BRCA2, while abundance of two other HDR proteins (RAD51 and

RPA70) remained unchanged. Because HDR proteins are recruited to discrete nuclear foci in response to DSB, both formation and resolution of HDR protein foci were monitored by immunofluorescence, which demonstrated that BRCA1 and BRCA2 foci resolution, but not RPA70 foci, were perturbed by beta-HPV 5 and 8 E6. To directly assay repair of DSBs, the investigators turned to a well-established assay that generates functional green fluorescent protein only when a DSB is repaired by the HDR pathway. These experiments corroborated previous results showing that beta-HPV 5 and 8 E6 indeed attenuate HDR. Finally, a role for p300 was queried by assaying both formation and resolution of HDR foci, as well as persistence of DSB markers, in p300 knockout cells. Strikingly, p300 knockout cells displayed similar defects to beta-HPV E6-expressing cells and expression of a degradation-resistant p300 restored DSB repair to normal levels. "We show that HPV 8 E6 further escalates the mutagenic potential of UV exposure by blocking expression of BRCA1 and 2, quintessential proteins for repairing double strand breaks. As a result, HPV 8 E6 expression can transform a fairly innocuous DNA lesion into one capable of inciting large-scale disruption of the human genome. I am proud to say that during my time at the Center, we have found significant support for the theory that some HPV infections can increase the risk of UV exposure," said Dr. Wallace. In conclusion, this study provides important mechanistic insights into how beta-HPV viruses act as accomplices in skin cancer.

Wallace NA, Robinson K, Howie HL, Galloway DA. 2015. beta-HPV 5 and 8 E6 Disrupt Homology Dependent Double Strand Break Repair by Attenuating BRCA1 and BRCA2 Expression and Foci Formation. *PLoS Pathog* 11(3):e1004687.

See also:

Wallace NA, Robinson K, Howie HL, Galloway DA. 2012. HPV 5 and 8 E6 abrogate ATR activity resulting in increased persistence of UVB induced DNA damage. *PLoS Pathog* 8(7):e1002807

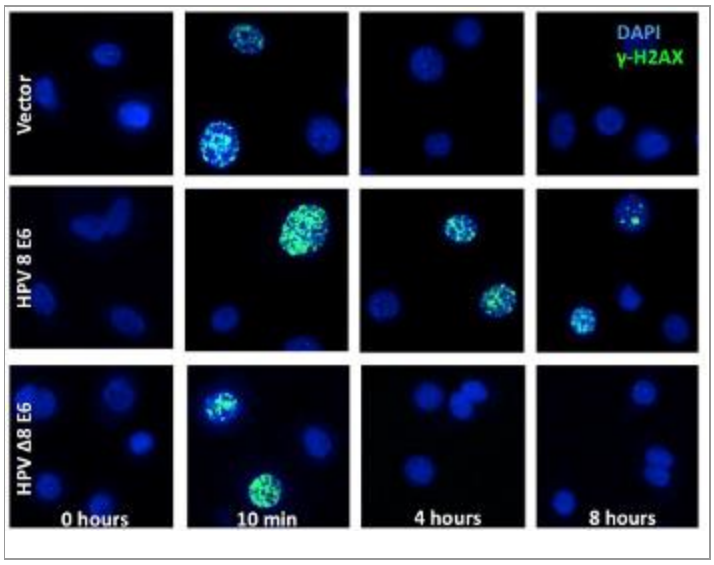

Image provided by Dr. Nicholas Wallace

Expression of the E6 protein from beta human papillomavirus type 8 (8E6, middle row) in primary human cells impairs the cell's ability to repair double strand breaks, increasing the time needed to remove these lesions (shown here as green nuclear foci by immunostaining for gamma-H2AX, a marker of double strand breaks). This attenuated repair is dependent upon the ability of 8E6 to promote the degradation of p300, a protein involved in the cellular response to DNA damage, as a mutation in HPV 8E6 (delta8e6, bottom row) that prevents p300 degradation also abrogates the disruption of DNA repair.