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No evidence for integrated viral DNA in the genome sequence of cutaneous squamous cell carcinoma

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To the Editors:

Cutaneous squamous cell carcinoma (cSCC) is the second most common type of non-melanoma skin cancer and is dramatically increased in the setting of immunosuppression such as organ transplantation (Grulich *et al.*, 2007; Hartevelt *et al.*, 1990; Jensen *et al.*, 1999; Lindelof *et al.*, 2000). This magnitude of increase is similar to that seen in virus-related cancers, such as human herpesvirus-8 mediated Kaposi's sarcoma and Epstein-Barr virus related lymphoma (Vajdic *et al.*, 2006). There have been multiple attempts to define a viral etiology for cSCC and to link cSCC with human papillomavirus (HPV). There is a clear epidemiologic association between HPV and cSCC (Aldabagh *et al.*, 2012), but detection has been limited to viral DNA. Two studies have demonstrated the absence of viral transcription in cSCC RNAseq data, confirming that direct viral transformation of keratinocytes is not required for maintenance of tumor (Arron *et al.*, 2011; Ganzenmueller *et al.*, 2012). These data suggest that, if there is a viral pathogen causing cSCC, the mechanism of carcinogenesis is not simply through expression of viral oncogenes.

This raises the possibility of alternate mechanisms of oncogenesis, including insertional mutagenesis, in which viral integration into the genome disrupts human genes of cell cycle regulation (Leib-Mosch *et al.*, 1990). This would be unlikely for a papillomavirus, but high-risk α-papillomaviridae do aberrantly integrate as part of cervical oncogenesis (Wentzensen *et al.*, 2004), and it is possible that cutaneous β-papillomaviridae do the same. Alternately, DNA from a previously undetected virus may be integrated into the genome or persist as extragenomic DNA. We used a newly published software package, Integrated Metagenomic Sequence Analysis (IMSA) (Dimon *et al.*, 2013), to address the hypothesis that HPV or another virus may be integrated into the cSCC genome or otherwise present in cancer DNA.

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Conflict of Interest

The authors state no conflict of interest.

Exome sequence from 12 cSCC and patient-matched normal skin were obtained from a previously reported study (Durinck *et al.*, 2011). Exome sequence from the MCF7 breast cancer cell line was used as a negative control. Previously described WGS data from SiHa and CaSki cell lines were used as positive controls for viral analysis (Conway*et al.*, 2012).

Pathogen analysis was performed with IMSA (Dimon *et al.*, 2013). Low quality and human reads were filtered against the human genome (hg19) and non-human reads were aligned to the NCBI nucleotide database. An average of 9.4×10^7 reads were obtained from each sample; 2.4×10^5 non-human reads remained after IMSA filtering (0.25%). Non-human and intronic reads in exome datasets result from incomplete capture efficiency. In order to confirm our ability to detect potential pathogen reads in exome datasets, we measured the percent of aligned reads on target using Picard. Picard is a set of standard bioinformatics software tools for high throughput sequence analysis. The tools are open source and can be found at http://picard.sourceforge.net/. The specific tool that we used was CalculateHsMetrics, which is designed to measure the efficiency of targeted sequencing such as exome sequencing. Capture efficiencies ranged between 66.1% and 78.1%. On average 27.3% of the reads in each dataset were non-exome. These libraries were sequenced to great depth for the purpose of cancer mutation analysis, therefore ample sequence was available for metagenomics.

IMSA generates a quantitative score for each taxonomic node, reflective of the level of nonhost DNA. To compare between datasets, we normalized the taxonomy score per million reads. cSCC had more bacterial reads on average than normal skin (65% vs 33% of nonhuman reads, Figure 1). 16% of cSCC and 30% of normal nonhuman reads mapped to vertebrates, most commonly nonhuman primate. These likely represent human DNA with sufficient divergence or sequencing errors to pass through the filters.

1% or fewer reads mapped to virus (Figure 2). The median normalized score for cSCC samples was 0.26 (IQR 0.1-0.7), no different from normal tissue (0.24, IQR 0.06-0.9, paired t-test p=0.3). In contrast SiHa had a score of 6.25 and CaSki had a score of 1272.6, derived exclusively from HPV16 reads.

One exome set contained significantly higher viral read scores, 6.2 in tumor and 36.3 in normal skin. These reads were derived from torque teno virus (TTV). TTV is a ubiquitous 3.8Kb circular single stranded DNA virus that has not been associated with disease. TTV viremia reflects immune function (Moen *et al.*, 2003), and this tumor-normal pair was from an immunosuppressed lung transplant recipient. Two of these reads indicated integration into the human genome.

To maximize the probability of detecting pathogen, we also examined WGS from three tumor-normal pairs. Whole genome sequence (WGS) libraries were prepared with the NuGEN Ovation Ultralow Library System and sequenced with the Illumina sequencing-by-synthesis platform. 3×10^8 reads were obtained from each WGS readset; with over 2×10^6 non-human reads remaining after IMSA filtering. The tumor-normal pairs had normalized viral read scores within the background range; 0.04/0.06, 0.24/0.08, and 0.01/0.05, (p=0.9, 95%CI -0.6-0.6) (Figure 2).

These data demonstrate the absence of viral DNA, including HPV DNA, in the exome and WGS of cSCC. This is consistent with prior qPCR data demonstrating extraordinarily low HPV DNA copy number in cSCC (typically less than 1 copy/100 cells) (Arron *et al.*, 2011) and with the newly developing consensus that viral infection is not required for tumor maintenance. In contrast, viral DNA was readily detected in two cervical cancer cell lines, CaSki and SiHa.

Despite these findings, the consistent association of HPV DNA with cSCC remains unclear. One remaining possibility is a hit and run mechanism, by which the virus destabilizes the keratinocyte until sufficient UV-mediated DNA mutations accumulate for transformation. This proposed role for viral tumor induction would be compatible with existing sequencing data, which has been limited to invasive tumor tissue.

It is possible that virus plays no role in cSCC but is rather a marker of cutaneous immunosuppression. This would explain the predilection of HPV for sun-exposed skin and the increased prevalence of HPV in the skin of immunosuppressed patients (Aldabagh *et al.*, 2012). Our finding of TTV in the tumor and adjacent normal skin of a lung transplant recipient suggests that immunosuppression predisposes to unrelated viral carriage.

It remains imperative to determine whether cSCC is a viral cancer, as this would have major implications for patient risk assessment and screening. Identifying a causative virus would open avenues for improved detection, targeted molecular therapies, and a potential vaccine for cancer prophylaxis. Additional studies may continue to shed light on this controversial and important subject.

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Abbreviations

cSCC	cutaneous squamous cell carcinoma
HPV	human papillomavirus
IMSA	integrated metagenomic sequence analysis
TTV	Torque teno virus
WGS	Whole genome sequencing

References

Aldabagh B, Angeles JG, Cardones AR, Arron ST. Cutaneous Squamous Cell Carcinoma and Human Papillomavirus: Is There an Association? Dermatol Surg. 2012

Arron ST, Ruby JG, Dybbro E, Ganem D, Derisi JL. Transcriptome Sequencing Demonstrates that Human Papillomavirus Is Not Active in Cutaneous Squamous Cell Carcinoma. J Invest Dermatol. 2011

Conway C, Chalkley R, High A, Maclennan K, Berri S, Chengot P, et al. Next-generation sequencing for simultaneous determination of human papillomavirus load, subtype, and associated genomic copy number changes in tumors. J Mol Diagn. 2012; 14:104–11. [PubMed: 22240447]

Dimon MT, Wood HM, Rabbitts PH, Arron ST. IMSA: Integrated Metagenomic Sequence Analysis for Identification of Exogenous Reads in a Host Genomic Background. PLoS ONE. 2013; 8

Durinck S, Ho C, Wang NJ, Liao W, Jakkula LR, Collisson EA, et al. Temporal Dissection of Tumorigenesis in Primary Cancers. Cancer Discov. 2011; 1:137–43. [PubMed: 21984974]

Ganzenmueller T, Yakushko Y, Kluba J, Henke-Gendo C, Gutzmer R, Schulz TF. Next-generation sequencing fails to identify human virus sequences in cutaneous squamous cell carcinoma. Int J Cancer. 2012; 131:E1173–9. [PubMed: 22488422]

Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007; 370:59–67. [PubMed: 17617273]

Hartevelt MM, Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in The Netherlands. Transplantation. 1990; 49:506–9. [PubMed: 2316011]

Jensen P, Hansen S, Moller B, Leivestad T, Pfeffer P, Geiran O, et al. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. J Am Acad Dermatol. 1999; 40:177–86. [PubMed: 10025742]

Leib-Mosch C, Brack-Werner R, Salmons B, Schmidt J, Strauss PG, Hehlmann R, et al. The significance of retroviruses in oncology. Onkologie. 1990; 13:405–14. [PubMed: 2092276]

Lindelof B, Sigurgeirsson B, Gabel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. Br J Dermatol. 2000; 143:513–9. [PubMed: 10971322]

Moen EM, Sagedal S, Bjoro K, Degre M, Opstad PK, Grinde B. Effect of immune modulation on TT virus (TTV) and TTV-like-mini-virus (TLMV) viremia. J Med Virol. 2003; 70:177–82. [PubMed: 12629661]

Vajdic CM, McDonald SP, McCredie MR, van Leeuwen MT, Stewart JH, Law M, et al. Cancer incidence before and after kidney transplantation. JAMA. 2006; 296:2823–31. [PubMed: 17179459]

Wentzensen N, Vinokurova S, von Knebel Doeberitz M. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. Cancer Res. 2004; 64:3878–84. [PubMed: 15172997]

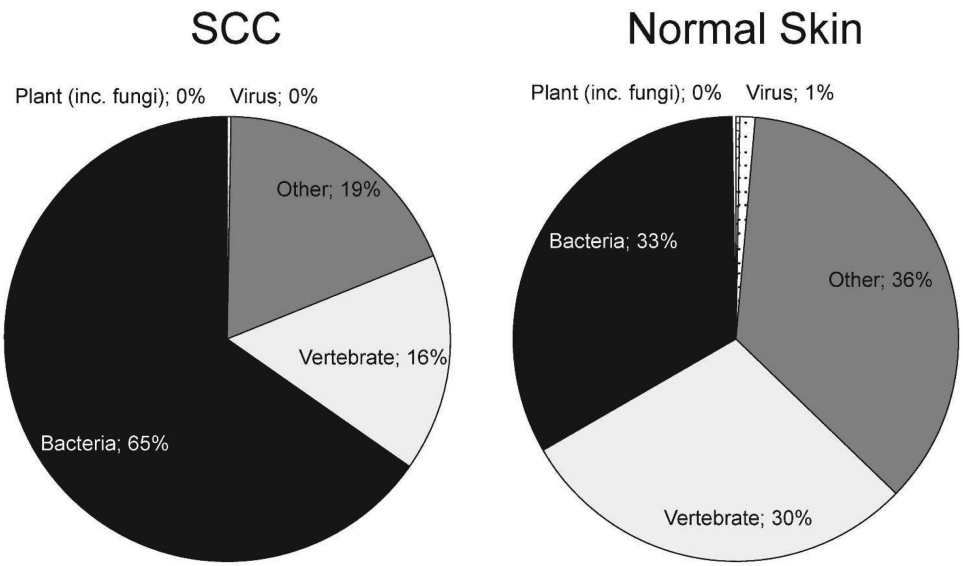

Figure 1.

Taxonomic classification of nonhuman reads in cSCC and normal skin. The percents shown are rounded to the nearest integer. In viral samples, the average percent viral reads is 0.145% while in normal skin the average percent viral reads is 1.139%.

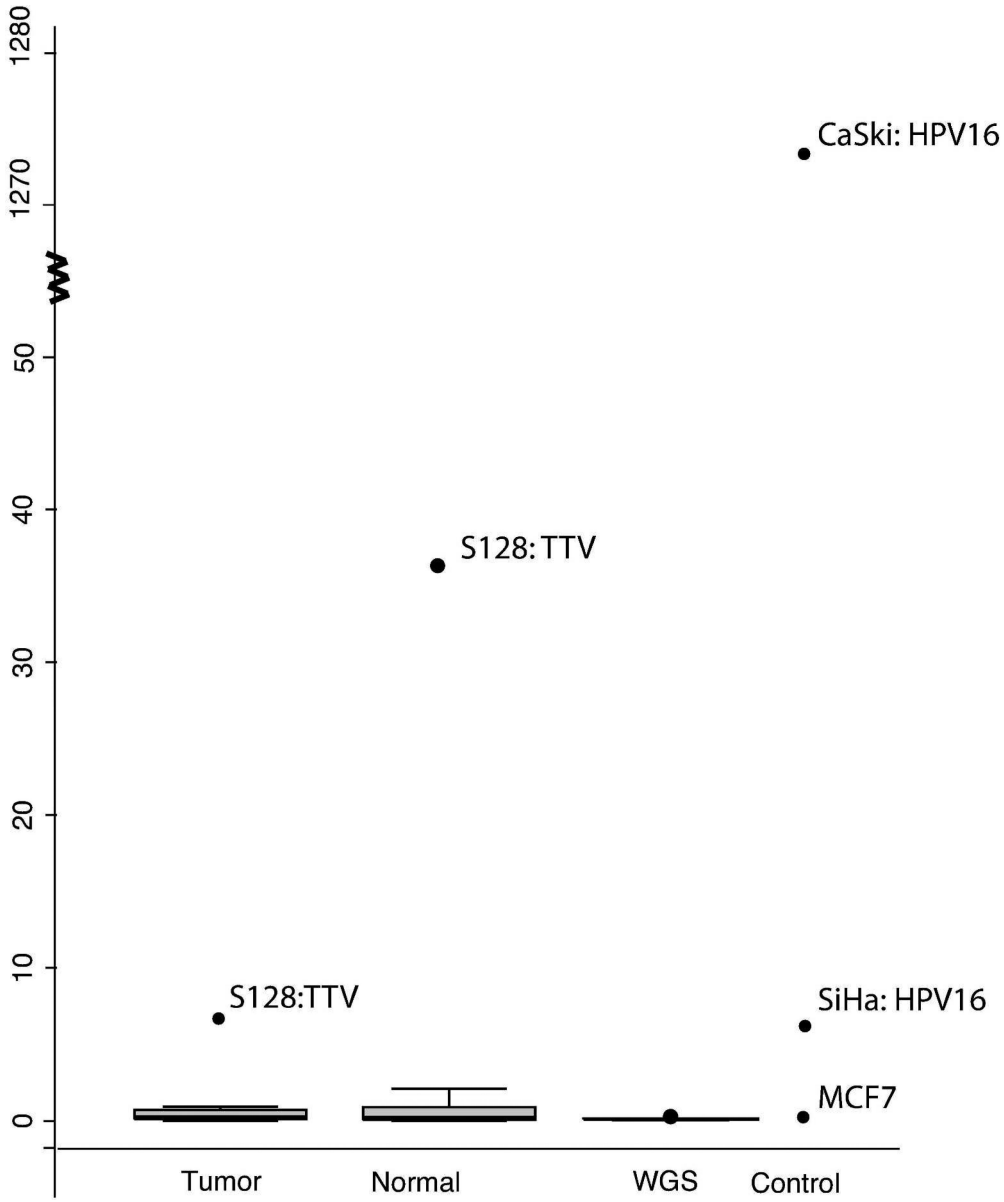

Figure 2.
Viral sequence scores per million sequence reads.