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ORIGINAL ARTICLE

Mutational signatures and chromosome alteration profiles of squamous cell carcinomas of the vulva

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Vulvar squamous cell carcinoma (SCC) consists of two different etiologic categories: human papilloma virus (HPV)-associated (HPV (+)) and HPV-non-associated (HPV (−)). There have been no genome-wide studies on the genetic alterations of vulvar SCCs or on the differences between HPV (+) and HPV (−) vulvar SCCs. In this study, we performed whole-exome sequencing and copy number profiling of 6 HPV (+) and 9 HPV (−) vulvar SCCs and found known mutations (*TP53*, *CDKN2A* and *HRAS*) and copy number alterations (CNAs) (7p and 8q gains and 2q loss) in HPV (−) SCCs. In HPV (+), we found novel mutations in *PIK3CA*, *BRCA2* and *FBXW7* that had not been reported in vulvar SCCs. HPV (−) SCCs exhibited more mutational loads (numbers of nonsilent mutations and driver mutations) than HPV (+) SCCs, but the CNA loads and mutation signatures between HPV (+) and HPV (−) SCCs did not differ. Of note, 40% and 40% of the 15 vulvar SCCs harbored *PIK3CA* and *FAT1* alterations, respectively. In addition, we found that the SCCs harbored kataegis (a localized hypermutation) in 2 HPV (+) SCCs and copy-neutral losses of heterozygosity in 4 (one HPV (+) and 3 HPV (−)) SCCs. Our data indicate that HPV (+) and HPV (−) vulvar SCCs may have different mutation and CNA profiles but that there are genomic features common to SCCs. Our data provide useful information for both HPV (+) and HPV (−) vulvar SCCs and may aid in the development of clinical treatment strategies.

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INTRODUCTION

Vulvar cancer is a malignant invasive lesion occurring in the vaginal opening, the labia majora (the most common site), the labia minora and the clitoris. Vulvar cancer accounts for 0.6% of all cancer diagnoses and 5% of gynecologic cancers.[1,2] Vulvar cancer is typically a squamous cell carcinoma (SCC) that is also common in other gynecologic organs, including the cervix and vagina.[1,2] Whereas almost all cervical SCCs occur with the background of human papillomavirus (HPV) infection,[3,4] vulvar SCCs consist of those associated with HPV as well as others independent of HPV infection.[1,2] Both HPV (+) and HPV (−) vulvar SCCs are preceded by vulvar intraepithelial neoplasia (VIN).[5,6] These two types of vulvar SCCs along with their precursors exhibit different epidemiological, pathological, clinical and molecular features.[1,2,5,6] Approximately one-third of all vulvar SCC patients suffer from recurrence, for which therapeutic options are limited.[7]

SCCs developed in female genital tracts share common features, including HPV infection and the progression of squamous intraepithelial neoplasia to invasive SCCs.[3,4] Like cervical intraepithelial neoplasia, VINs progress to SCCs but the progression rates are lower than those of cervical lesions,[5,6] indicating that the pathogenesis of cervical and vulvar SCCs may differ in part.

With the improvement of next-generation sequencing (NGS) technology, comprehensive molecular profiles of many cancers have been studied using NGS,[8] which allows for the

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investigation of thousands of variants within a given tumor sample.[9–12] Using a targeted sequencing approach for 14 genes, a recent study identified that most HPV-negative (−) vulvar SCCs (83%) contained one or more somatic mutations in *TP53*, *CDKN2A*, *HRAS*, *KRAS*, *PIK3CA*, *PPP2R1A* and *PTEN*, with *TP53* being the most commonly mutated gene.[13] In contrast, HPV-positive (+) vulvar SCCs harbored a *TP53* mutation in 17% of cases but the remaining 83% were silent without any driver mutations.[13] Their study strongly suggests not only that HPV-dependent and HPV-independent vulvar SCCs may have different mutation profiles but also there could be hidden mutations not discovered by the targeted approach only for the 14 genes. Other studies using conventional gene-to-gene analyses have shown similar mutation data in vulvar SCCs.[1,2,14,15] Thus far, a number of studies have reported the mutational profiles of SCC in many organs (non-genital: head/neck, esophagus and skin; genital: uterine cervix and penis) using high-throughput genome profiling technologies.[11,16–19] However, to date, the genomic data of vulvar SCCs at the whole-genome or whole-exome level is absent. Thus, it is necessary to examine the mutational profiles of vulvar SCCs in addition to the well-known gene mutations, including *TP53*. In this study, we analyzed the genomes of 15 vulvar SCCs using NGS-based whole-exome sequencing (WES) to identify the mutational profiles of vulvar SCCs.

MATERIALS AND METHODS

Vulvar cancer tissues

Vulvar SCC tissues resected by surgery were obtained from 15 Korean patients. Tissues from one patient were frozen (VSCC2) and those from the other 14 patients were formalin-fixed paraffin-embedded (FFPE). This study was approved by the Institutional Review Board (IRB) at The Catholic University of Korea, College of Medicine. Clinicopathologic characteristics of the 15 vulvar SCC patients are summarized in Table 1. Both frozen and FFPE tissues were cut and examined under a microscope by two pathologists. Tumor and normal cells were selectively procured from hematoxylin-stained sections by microdissection. The purity of the tumor cells from the microdissection was approximately 70%. HPV (+) tumors were determined using real-time polymerase chain reaction (PCR) for HPV obtained from a commercial company for molecular diagnosis (Seegene, Inc., Seoul, Korea).

WES and somatic mutations

WES was performed on the genomic DNA obtained from tumor and matched normal tissues using the Agilent SureSelect Human All Exome V4 Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. WES data were processed as previously described.[20] Briefly, the paired-end sequencing raw data (FASTQ format) were aligned to the human reference genome (hg19) using Burrows–Wheeler aligner software (BWA, v0.7.15).[21] The Genome Analysis toolkit (GATK, v3.6),[22] Picard (http://picard.sourceforge.net; v2.7.1) and Samtools (v1.3.1)[23] were used for the basic processing and management of marking duplicates, including local realignments and score recalibration. Somatic mutations were detected by comparing tumor and matched normal sequencing data using MuTect[24] and SomaticIndelDetector[22] for point mutations and indels, respectively. ANNOVAR (Annotate Variation) was used for the functional annotation of each variant in the coding regions.[25] PolyPhen-2 was used to predict the impact of an amino-acid substitution on protein function and structure.[26] We validated 21 mutations of 9 genes using either digital PCR or Sanger sequencing. Putative regions of kataegis were identified in samples where mutations had an average inter-mutation distance of no more than 10 kb.[27]

Comprehensive analysis of mutational signatures

Somatic mutation signatures were estimated using the SomaticSignatures R package,[28] in which a mutation spectrum was decomposed with a non-negative matrix factorization (NMF) algorithm. Mutational signature analyses were rerun 20 times to confirm the results

Table 1 Clinicopathologic features of vulvar cancers

Sample	*Age (years)*	*Tumor size in diameter (cm)*	*Diagnosis*	*Extent of carcinoma*	*HPV infection (high- and low-risk types)*	*Sample source*
VSCC1	60	3.5	Vulva SCC	Microinvasion	+ (HPV16, −)	FFPE
VSCC2	74	3	Vulva SCC	invasion (7.0 mm)	+ (HPV16, −)	Frozen
VSCC3	78	3	Vulva SCC	Invasion (8.0 mm)	+ (HPV58, −)	FFPE
VSCC4	61	0.5	Vulva SCC	Microinvasion	+ (HPV16, −)	FFPE
VSCC5	66	2	Vulva SCC	Invasion (6.0 mm)	+ (HPV52, −)	FFPE
VSCC6	47	1.5	Vulva SCC	Invasion (1.0 mm)	+ (HPV16, −)	FFPE
VSCC7	70	2.5	Vulva SCC	Invasion (7.0 mm)	—	FFPE
VSCC8	70	4	Vulva SCC	Invasion (4.0 mm)	—	FFPE
VSCC9	45	1.3	Vulva SCC (VIN)	No invasion	—	FFPE
VSCC10	61	0.6	Vulva SCC	Invasion (1.4 mm)	—	FFPE
VSCC11	62	2.4	Vulva SCC	Invasion (1.0 mm)	—	FFPE
VSCC12	69	4	Vulva SCC	Invasion (7.0 mm)	—	FFPE
VSCC13	79	4	Vulva SCC	Invasion (1.0 mm)	—	FFPE
VSCC14	64	1.7	Vulva (VIN) SCC	No invasion	—	FFPE
VSCC15	83	1.2	Vulva SCC	Invasion (4.0 mm)	—	FFPE

Abbreviations: FFPE, formalin-fixed paraffin-embedded; HPV, human papilloma virus; VIN, vulva intraepithelial neoplasm.
HPV infection risk types are indicated in parentheses (high risk, low risk).
High-risk HPVs are indicated by the numbers 16, 52, and 58.

and an optimal number of signatures was chosen based on the maximum differentiation between the signatures. To compare the mutation signatures of the 15 vulvar SCCs with other cancer types, we first calculated the similarities between the mutational signatures using 30 known signatures recorded in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://cancer.sanger.ac.uk/cosmic/signatures).[29] Similarities between mutational signatures were calculated using a cosine correlation similarity ranging from 0 and 1, where one represented identical signatures and zero represented completely non-identical signatures. Next, we systematically analyzed the mutational signatures of SCCs using data from The Cancer Genome Atlas (TCGA)[30] and a comprehensive literature review of NGS-based studies for SCCs.[11,16,31–33] Data were composed of four types of SCCs from TCGA (CESC: cervical squamous cell carcinoma, ESCA: esophageal squamous cell carcinoma, HNSC: head and neck squamous cell carcinoma, LUSC: lung squamous cell carcinoma)[30] and two SCCs from NGS-based studies (CSCC: skin cutaneous squamous cell carcinoma,[11,16] OSCC: oral squamous cell carcinoma[31–33]).

DNA copy number and loss of heterozygosity analyses

Copy number alterations (CNAs) were defined for each of the 15 vulvar SCCs using the ngCGH module and RankSegmentation statistical algorithm in NEXUS software v7.5 (Biodiscovery, Inc., El Segundo, CA, USA).[34] Loss of heterozygosity (LOH) events were inferred using Sequenza.[35] Identified CNAs and LOH events were manually curated based on the depth ratio and B allele frequency.

RESULTS

The catalog of somatic mutations

A total of 15 vulvar SCC genomes (6 HPV (+) and 9 HPV (−)) and paired normal tissue genomes were analyzed in this study (Table 1). The coverage of the sequencing depth was a