UNIVERSIDAD AUTÓNOMA DE GUERRERO
UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS
DOCTORADO EN CIENCIAS BIOMÉDICAS

“Expresión global de genes por efecto de la oncoproteina E6 de variantes del VPH 16 en células de cáncer cervical”

T E S I S

Que para obtener el grado de

Doctor en Ciencias Biomédicas

P R E S E N T A

M en C. ANA ELVIRA ZACAPALA GÓMEZ

DIRECTORA DE TESIS:
Dra. Berenice Illades Aguiar

CODIRECTOR DE TESIS:
Dr. Oscar Del Moral Hernández

ASESOR EXTERNO:
Dr. Nicolás Villegas Sepúlveda

CHILPANCINGO, GRO. DICIEMBRE DEL 2015.

UNIVERSIDAD AUTÓNOMA DE GUERRERO
UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS
UNIDAD DE INVESTIGACIÓN ESPECIALIZADA EN MICROBIOLOGÍA
DOCTORADO EN CIENCIAS BIOMÉDICAS

ACTA DE APROBACIÓN DE TESIS

En la ciudad de Chilpancingo, Guerrero, siendo los 01 días del mes de junio del dos mil quince, se reunieron los miembros del Comité Tutoral designado por la Academia de Posgrado del Doctorado en Ciencias Biomédicas, para examinar la tesis titulada **"Expresión global de genes por efecto de la oncoproteína E6 de variantes del VPH 16 en células de cáncer cervical"**, presentada por la alumna Ana Elvira Zacapala Gómez, para obtener el Grado de Doctora en Ciencias Biomédicas. Después del análisis correspondiente, los miembros del comité manifiestan su aprobación de la tesis, autorizan la impresión final de la misma y aceptan que, cuando se satisfagan los requisitos señalados en el Reglamento General de Estudios de Posgrado e Investigación Vigente, se proceda a la presentación del examen de grado.

El Comité Tutoral

Dra. Berenice Illades Aguiar
Dirección de tesis

Dra. Luz del Carmen Alarcón Romero

Dr. Marco Antonio Leyva Vázquez

Dra. Gloria Fernández Tilapa

Dr. Nicolás Villegas Sepúlveda

Vo. Bo

Dra. Isela Parra Rojas
Coordinadora del Posgrado de la Unidad Académica de Ciencias Químico Biológicas

UNIVERSIDAD AUTONOMA DE GUERRERO
UAGro
Coordinación del Posgrado UACQB

UNIVERSIDAD AUTÓNOMA DE GUERRERO
UAGro
Unidad Académica de Ciencias Químico Biológicas
DIRECCIÓN 2014 - 2018

Vo. Bo

Dra. Amalia Vences Velázquez
Directora de la Unidad Académica de Ciencias Químico Biológicas

Coolaboraciones del proyecto

Este trabajo se realizó en el Laboratorio de Biomedicina Molecular de la Unidad Académica de Ciencias Químico Biológicas de la Universidad Autónoma de Guerrero en Chilpancingo Guerrero.

Bajo la dirección de:
Dra. Berenice Illades Aguiar

Codirección de:
Dr. Oscar del Moral Hernandez

Asesor externo:
Dr. Nicolas Villegas Sepulveda

Y bajo la asesoría de:
Dra. Luz del Carmen Alarcón Romero
Dr. Marco Antonio Leyva Vázquez
Dra. Gloria Fernandez Tilapa

Durante el período en que cursó el Doctorado en Ciencias Biomédicas, la M en C. Ana Elvira Zacapala Gomez, recibió beca **CONACYT** (Febrero 2012-Enero 2015).

Se estableció colaboración con el Dr. Nicolás Villegas Sepúlveda, investigador de tiempo completo del Departamento de Biomedicina Molecular del CINVESTAV, para la realización de clonaciones, transformación y transfección. Además se estableció colaboración con el Dr. Alfredo Hidalgo Miranda jefe del Laboratorio de Genómica del Cáncer del INMEGEN para la realización de los microarreglos.

AGRADECIMIENTOS

A la Dra. Berenice Illades Aguiar
Por haber confiado en mí para realizar este gran proyecto, por esas platicas tan productivas en las cuales discutíamos sobre los resultados obtenidos, sobre cómo mejorar la tesis, sobre cómo podríamos organizar el trabajo del laboratorio para terminar la investigación, etc. Por permitirme vivir de cerca todo el proceso para la publicación del artículo. Por todas sus acertadas correcciones en la tesis y en el artículo. Por ser más que mi directora de tesis, por ser mí amiga y apoyarme en momento difíciles, tanto anímicamente como económicamente. Por todos los consejos brindados, es para mí un gran honor que la persona que admiro se tome el tiempo para platicar conmigo.

Dr. Oscar del Moral Hernández
Por todo su apoyo brindado, por dedicarme parte su tiempo y conocimientos para mejorar la tesis, por ayudarme a mejorar las presentaciones para los seminarios, por ayudarme con la redacción del artículo, por todas sus correcciones tan acertadas, por corregir mi vocabulario para decir correctamente términos científicos, por permitirme formar parte de su grupo de trabajo.

Dr. Nicolás Villegas Sepulveda
Por haberme recibido en su laboratorio, por todo el apoyo académico brindado, por su interés para que esto que solo era un proyecto se convirtiera en una realidad, por todas sus observaciones y apoyo para lograr la publicación del artículo, por la amabilidad y paciencia demostrada durante mi estancia en el Cinvestav

Biol. Raúl Bonilla Moreno
Por toda la paciencia para instruirme en el laboratorio, muchísimas gracias por transmitirme parte de sus conocimientos, por enseñarme la base de varios experimentos, por apoyarme cuando las cosas se tornaban difíciles, por esas horas de conversación sobre mi tesis. Infinitas gracias.

A los integrantes de mi comité tutoral, **Dra. Luz del Carmen Alarcón Romero, Gloria Fernández Tilapa y Dr. Marco Antonio Leyva Vázquez:** Por sus aportaciones y sugerencias para la mejora de la tesis. Por compartir parte de sus conocimientos

"Cada vez que trabajaba con usted terminaba mas enamorada de la investigación, cada uno es un gran investigador, espero algún día ser por lo menos algo próximo a lo que usted han logrado"

DEDICATORIAS

A mi familia:

A mi **mamá Melita Zacapala Gómez**. Por apoyarme en este sueño a pesar de que eso significara que estuviera lejos de casa, gracias por tu inmenso amor demostrado.

A mi hermana Sandra Guadalupe Zacapala Gómez. Por su apoyo incondicional para lograr esta meta, por los consejos otorgados para lograr salir de momentos difíciles, por todos los momentos vividos y por ser mi amiga.

José GonzálezTorrez. Por todo el apoyo otorgado para finalizar el doctorado, gracias por el interés mostrado para que todo culminara de la mejor manera.

A mi sobrino Erick Mahonry Velilla Zacapala: Por comprender a su corta edad (6 años) que a pesar de que estemos separados lo quiero mucho, por todos los momentos vividos, por esos momentos de hacer la tarea juntos, por apoyarme cuando tuvimos que venir a la escuela en horario extra.

A mis amigos (a):

Por brindarme su amistad, por apoyarme en este sueño y echarme porras, por darme la mano en aquellos momentos en que me sentía mal, por no dejarme vencer, por soportar mi estrés en momentos desesperantes, por compartir alegrías y tristezas conmigo, por comprender mi ausencia en ocasiones, por escucharme, etc., no me alcanzarían las palabras para agradecer.

Central de laboratorio: Abuelita Hilda, Abuelito Julio, Tío Javier, Papi Manuel, Hugo, gemela Quetzali, Anaid.

Laboratorio de biomedicina: Irlanda, Hugo, Israel, Jaqueline, Jazmín, Genaro, Olga, Monse, Eduardo, Ramón, Luis, Tania, Josué, Alinne, Naty, Dra. Olga, Dr. Vero, M en C. Noelio, Dr. Migue, Dr. Daniel.

Amigos y compañeros de generación: Linda Anahí, Cahua, Zuby, Pepe, Inés

Amigos y roming: Aby, Ivette, Jorge Ocampo, Isaura, Hoguer

Amigos e hijos académicos: David y Arturo

Amigos del Cinvestav: Carolina, Macario, Job, Raúl, Leti, Carmen, Manuel, Mary Carmen, Mony, Mary, Paola Carolina, Julio, Sofi, Eloy, Víctor.

Amigos desde la licenciatura: Agus, Efra, Angélica, Brenda, Janet, Javier Flores, Chayo, Lizz Mondragón, Evelin, Victor Manuel, Chio Arellano, Pedro Pablo

Amigos: Cruz, Nelissa, Jorge Barrera, Casar, Paola Pérez, Pao, Rocio Almazan, Carlos.

Los quiero mucho. Gracias por todo lo vivido.

“Expresión global de genes por efecto de la oncoproteina E6 de variantes del VPH 16 en células de cáncer cervical”

ÍNDICE

Resumen

Introducción: Se ha reportado que las variantes del VPH 16 tienen diferente potencial oncogénico, algunos reportes muestran que las variantes pueden regular la expresión de genes, sin embargo, no se ha analizado la expresión del transcriptoma completo por efecto de las variantes del VPH 16. **Objetivo:** Analizar el transcriptoma humano completo de células C33-A que expresan establemente a E6 de las variantes AA-a, AA-c, E-A176/G350, E-C188/G350 y E-G350 del VPH 16 vs. E6 de E-Prototipo del VPH 16. **Metodología:** Se transfectaron establemente células C33-A con E6 de las variantes de estudio, se realizó extracción de RNA, se realizaron microarreglos de mRNA (Affymetrix GeneChip Human Transcriptome Array 2.0 platform) que analiza la expresión de más de 245,000 transcriptos, se validaron los microarreglos con 7 sondas mediante qPCR. **Resultados:** La expresión de E6 de las variantes alteraron la expresión de 387 genes diferentes a los genes alterados por E6 de E-prototipo, de los cuales 229 genes son alterados por la expresión de E6 de la variante AA-c. Los genes que alteran su expresión por la expresión de E6 de las variantes participan en procesos celulares relacionados con el desarrollo de cáncer cervical: señalización (20.3%), transcripción (16.7%), adhesión (14.6%), proliferación (12.5%), apoptosis (9.4%), Migración (7.3%). Mediante un análisis de “*enrichment score*” se encontro que los genes que alteran su expresión por la expresión de E6 de la variante AA-a se asocian con adhesión celular, vias de señalización tirosin cinasa y actividad de proteínas cinasas; esta es la única variante capaz de alterar estos procesos. **Conclusión:** Los cambios de aminoácidos en E6 de las variantes del VPH 16 modulan la transcripción de genes específicos y cada variante parece tener su propia firma molecular.

Introducción

Los VPH son un grupo heterogéneo de virus de ADN, lo cuales difieren en su potencial oncogénico. (Lazarczyk *et al.,* 2009, Doorbar 2005). Los VPH son parte de la familia *papillomaviridae,* son virus pequeños, no envueltos, presentan un diámetro de 55 nm, su ADN de doble cadena esta conformado de 8,000 pares de nucleótidos que dependiendo del genotipo específico del VPH pueden contener de 7 a 9 marcos de lectura abierto (ORF). El ADN viral se asocia con histonas celulares para formar "minicromosomas" y es empaquetado en una cápside icosáedrica que contiene 72 capsómeros (Lazarczyk *et al.,* 2009).

El genoma del VPH se divide en tres regiones: región larga de control (LCR o UTR), región de proteínas tempranas (E1 a E7) y región de proteínas tardías de la cápside (L1 y L2). Cada proteína realiza funciones diferentes para llevar acabo la patogénesis (Tabla 1) (Doorbar 2007, Muñoz *et al.,* 2006).

Para que se lleve a cabo la infección, el VPH entra en las células del estrato basal por ruptura de la integridad de la lamina epitelial (Doorbar 2007, Woodman *et al*., 2007). El VPH se asocia con proteoglicanos de la membrana celular, así como con receptores secundarios que facilitan la captación viral. El acceso de los VPH es mediante sitios donde las células del estrato estratificado y columnar se unen (zona de transformación cervical) (Doorbar 2007).

Después de la infección viral, el VPH replica su genoma en un bajo número de copias, esta replicación se lleva a cabo de forma episomal. La replicación del ADN viral se lleva a cabo con la maquinaria de replicación celular, la replicación del ADN viral y la replicación del ADN celular conllevan a la progresión del ciclo celular a través de la fase S. Las proteínas que se consideran necesarias en esta fase son E1 y E2, el promotor de diferenciación (PL) modula la expresión de E1 y E2 (Doorbar 2007).

El VPH necesita mantener la división celular y para ello, requiere retardar la diferenciación de las células pero no inhibir la por completo. Esta condición

Tabla 1. Función de las proteínas codificadas en el genoma del VPH

Proteína	Función
E1	Es una helicasa que se une al origen del ADN viral y recluta factores celulares del hospedero para la replicación del genoma viral (Sakakibara *et al*., 2011, Lazarczyk *et al.,* 2009).
E2	Es un regulador transcripcional de la expresión de los genes E6 y E7. E2 ayuda a que E1 helicasa se reclute en el origen y facilita la correcta segregación de genomas durante la división celular. E2 es requerida para el ensamblaje de viriones (Sakakibara *et al*., 2011, Lazarczk *et al.,* 2009, Doorbar 2007).
E4	Se expresa en la superficie del epitelio y se considera que tiene participa en la liberación de virus por que se asocia con citoqueratinas y con la sobre cornificación. La contribución de E4 en la oncogenecidad es asociada con CDK y con E2. (Doorbar 2007, Lazarczk *et al.,* 2009)
E5	Evade la respuesta inmune, interfiriendo con el transporte del antígeno por el MHC de clase I a la superficie celular, promueve el crecimiento independiente de anclaje y disrupción de uniones gap*. Estimula la proliferación celular dependiente de EGF (Suprynowicz *et al.,* 2010).Participa en la inmortalización celular (Lazarczk *et al.,* 2009).
E6	El supresor de tumor p53 es su principal blanco, sin embargo, E6 puede interaccionar con varias proteínas (descritas más adelante) (Garnett *et al.,* 2006, Doorbar 2005).
E7	Se asocia con pRB para de esta forma transactivar genes celulares involucrados en la replicación viral (ciclina A y E), permitiendo a las células de la capa basal mantener la replicación del ADN. También se une a desacetilasas de histonas, componentes del complejo de transcripción AP1 y de los inhibidores de CDK, p27 y p21 (Doorbar 2007).
L1	Proteína menor de la cápside, realiza el ensamblaje de viriones (Doorbar 2007)
L2	Proteína mayor de la cápside que se expresa después de L1 y permite el ensamblaje de partículas infecciosas en el estrato superficial del epitelio, L2 se acumula en estructuras conocidas como cuerpos PML y recluta a L1. (Doorbar 2007)

CDK: quinasa dependiente de ciclinas, **MHC de clase I:** complejo de histocompatibilidad mayor de clase I, **PML**: proteína de leucemia promielocítica (se encuentran en el nucleoplasma), ***uniones gap:** responsables de la comunicación célula-célula, **EGF:** factor de crecimiento epidermal

puede garantizar la replicación del ADN del VPH en la capa suprabasal y superior granular. E6 y E7 son oncoproteínas cruciales para mantener la proliferación en el estrato superior del epitelio. E7 promueve la progresión a la fase S en células diferenciadas, mientras que la proteína E6 evita la apoptosis. (Lazarczyk *et al.,* 2009).

La proteína E5 puede asociarse con protón ATPasa y retardar el proceso de acidificación endosomal, para favorecer el reciclaje del receptor del factor de crecimiento epidermal (EGFR) en la superficie celular. La consecuencia de esto es el incremento en la señalización del receptor mediada por EGF, esto se considera que contribuye al mantenimiento de la replicación (Doorbar 2007).

Posterior a la amplificación de ADN viral, en el estrato superior del epitelio el genoma viral es empaquetado (Lazarczyk *et al*., 2009). Las proteínas que conforman la cápside (L1 y L2) son expresadas únicamente en células que han sido sometidas a la amplificación genómica y que contienen en su citoplasma a E4. L1 y L2 son localizadas en los cuerpos de PML (proteína de la leucemia promielocítica), la expresión de L2 precede a la expresión de L1 y el ensamblaje de los virus ocurre cuando los capsómeros de L1 entran al núcleo y son recluidos en las estructuras PML por L2. La asociación de L1 y L2 requiere de secuencias hidrofóbicas cerca de la región C-terminal de la proteína, esta región se inserta en el centro del capsómero pentavalente L1, por uniones disulfuro se lleva a cabo la interacción proteína-proteína la cual es estable en la capa superior epitelial, lo anterior, contribuyen a la estabilidad de las partículas liberadas. La acumulación de altos niveles de proteínas estructurales del virión es retardada hasta que la célula alcanza el estrato superior del epitelio, para limitar la detección por el sistema inmune del hospedero (Doorbar 2007).

El VPH se puede mantener en forma episomal o integrado (o mixto), la integración ocurre cuando el ADN viral se rompe en el gen E2, eliminando la expresión de la proteína E2, de tal manera que al ser eliminado el regulador negativo de la expresión E6 y E7 se favorece la sobreexpresión de las oncoproteínas E6 y E7 (Woodman *et al.,* 2007). Varios estudios han mostrado una asociación entre la integración y el desarrollo de LEIAG (Lesiones Escamosas Intraepiteliales de Alto Grado) o cáncer cervical (Theelen *et al.,* 2010, Koskimaa *et al.,* 2010, Jiang *et al.,* 2009) (Figura 1). La sobreexpresión de E6 esta asociada con el desarrollo de cáncer, E6 participa en el desarrollo de cáncer debido a que puede interaccionar con **1)** proteínas que participan en la regulación de la transcripción y replicación del ADN, **2)** proteínas involucradas en apoptosis y evasión de la respuesta

inmune, **3)** proteínas involucradas con la organización epitelial y diferenciación, **4)** proteínas involucradas en adhesión célula-célula, polaridad y proliferación, **5)** proteínas con motivos de unión PDZ y **6)** proteínas involucradas en la reparación del ADN. Por lo anterior E6 es una proteína indispensable para la carcinogénesis (Garnett *et al.,* 2006, Lee *et al.,* 2004).

La oncoproteína E6 está compuesta de 151 aminoácidos (aa) (Nomine *et al.,* 2006), presenta 2 dominios uno N-terminal (1-80 aa) y uno C-terminal (81-151aa) unidos por 36 aa, cada uno de estos dominios está conformado por tres láminas beta (β1, β 2, β3), tres α hélices (α1, α2 y α3) y un dedo de zinc que contiene 1 motivo CXXC-X_{29}-CXXC, el zinc y las cisteínas están unidos por puentes bisulfuros. Los dominios N-terminal (E6N) y C-terminal (E6C) son homólogos excepto porque en la región E6C se presenta un bucle flexible (residuos 80-84) denominado lámina β0, además el dominio E6C está cargado positivamente, excepto por una región neutra que presenta zonas hidrofóbicas y algunos puntos negativos en las hélices α1 y α2, por el contrario, E6N presenta regiones ácidas y básicas (Figura 2) (Wise *et al.,* 2008, Zanier *et al.,* 2012, Mischo *et al.,* 2013).

La estructura de E6 del VPH 16 juega un papel trascendental para la unión de E6 al ADN y a sus proteínas celulares blanco (Ristriani *et al.,* 2000, Zanier *et al.,* 2012). Para catalizar eficientemente la degradación de p53 es necesario que los dos dominios de E6 se encuentren unidos (E6C-E6N), si E6 presenta una mutación F47R impedirá que los domios de E6 se unan y afectará la degradación de p53, mutaciones en E6N afectarán la degradación de p53 pero no la unión de E6 a E6AP, debido a que E6N se une a p53 y E6C se une a E6AP (Zanier *et al.,* 2012).

Actualmente varios estudios *in vitro* han identificado el dominio específico mediante el cual E6 puede unirse a sus proteínas celulares blanco. Eliminaciones o la presencia de polimorfismos en la secuencia de ADN que codifican para E6 y que se traduce en un cambio de aminoácido conducen a la pérdida de la interacción entre E6 y su proteína blanco (Tabla 2) (Liu *et al.,* 1999, Park *et al.,* 2001, Patel *et al*., 1999, Gao *et al*., 1999, Mantovani *et al.,* 2001). Un estudio *in silico* sustenta que algunos cambios de aminoácidos en E6 podrían conducir a

cambios en la función de E6. Se observó que existen conjuntos de aminoácidos importantes para que E6 lleve a cabo funciones específicas; los aminoácidos del 10-14 son importantes para la unión y degradación de p53, además de participar en la interaccion con celulas T y B; los aminoácidos del 25-29 son importantes para la interacción entre E6 y células T; los aminoácidos del 135-141 son importantes para la transactivación transcripcional de E6, unión/degradación de p53 y para la interacción de E6 a células B y T (Tabla 3) (Pillai *et al.,* 2009).

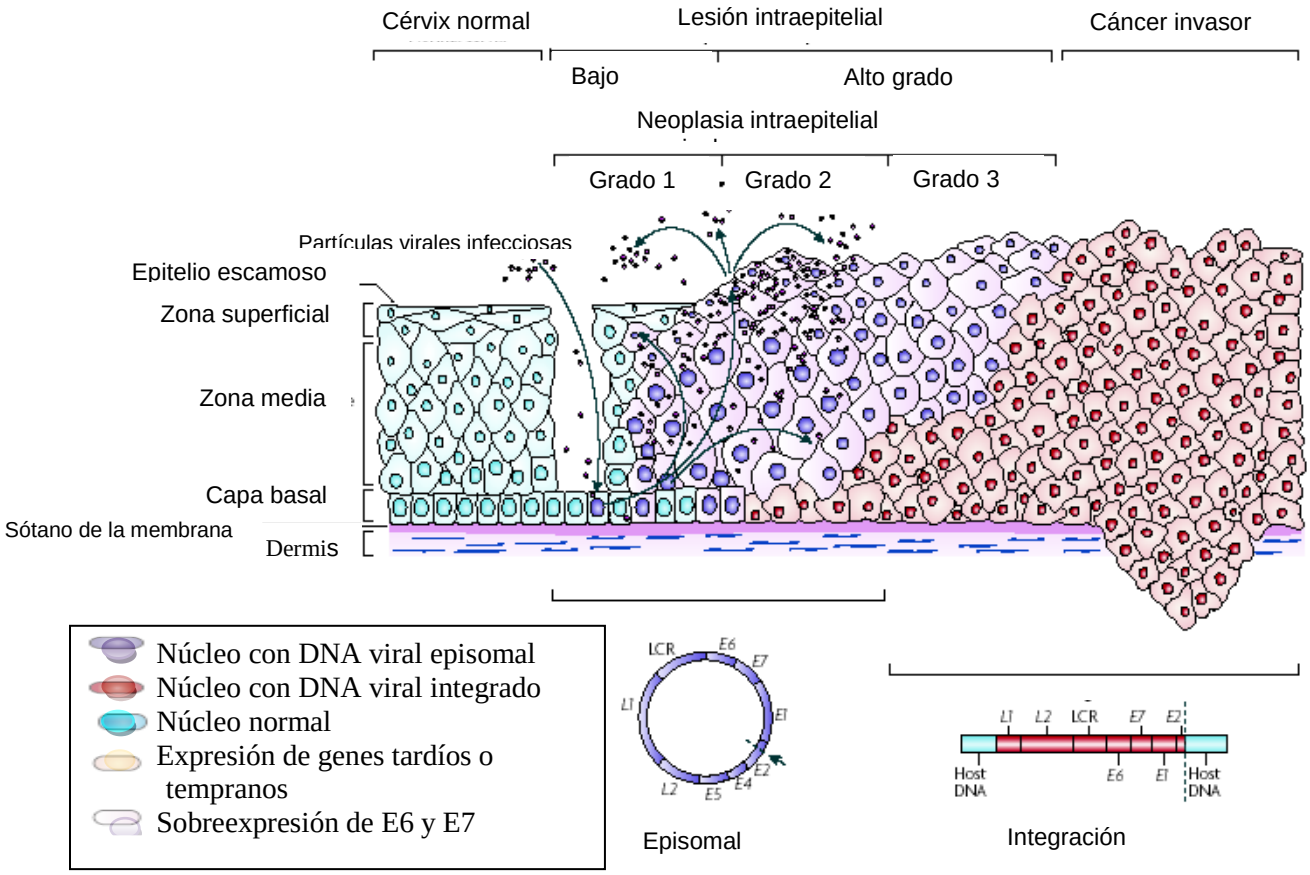


Figura 1. Progresión a cáncer cervical por infección con VPH. (Adaptada de Woodman *et al.,* 2007)

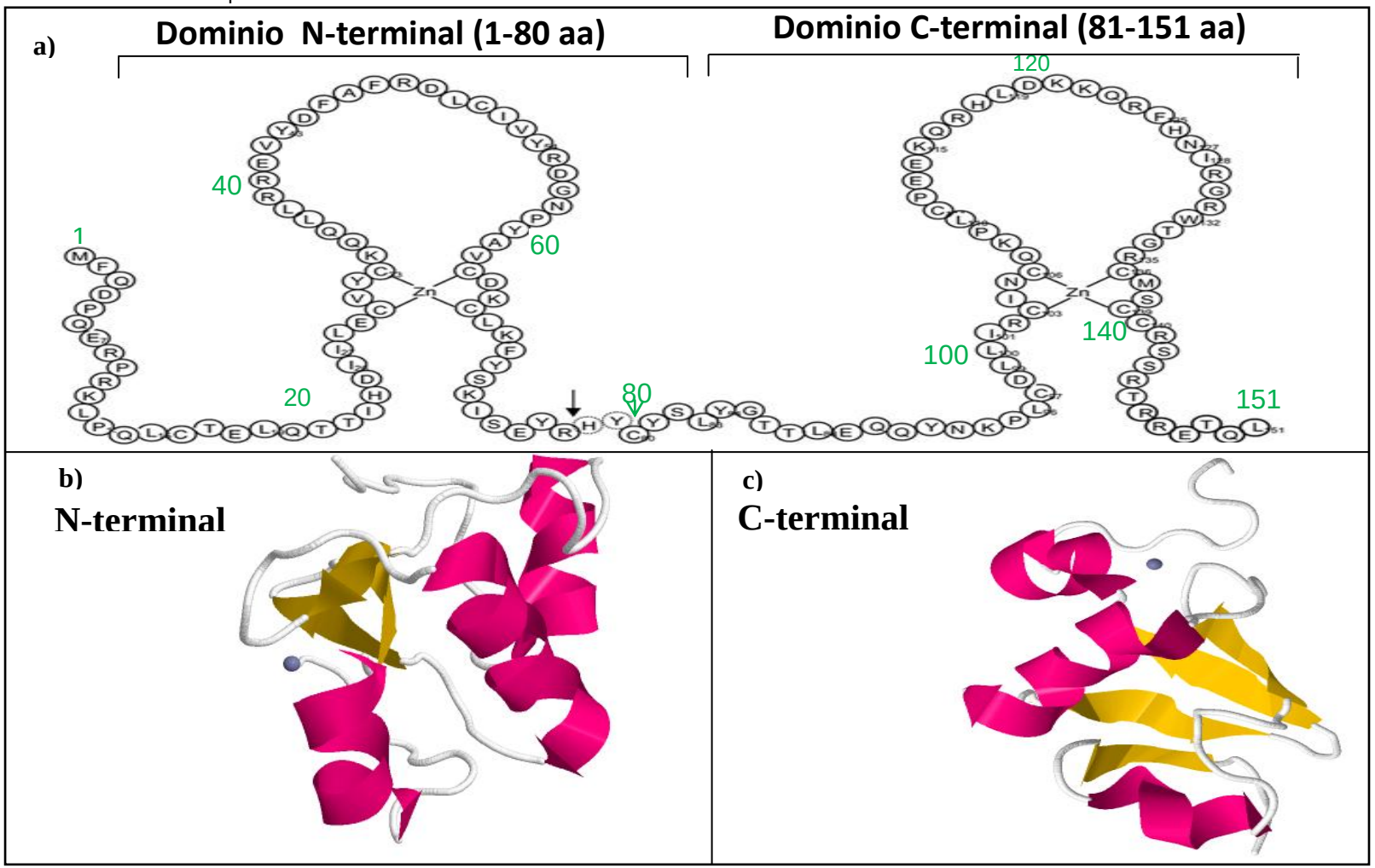


Figura 2. Estructura de E6 del VPH 16. a) Estructura secundaria de la proteína E6 del VPH 16, **b)** dominio N-terminal PDB: 2LJX, **c)** dominio C-terminal, PDB: 2LJZ. Los numeros en verde denotan el número del aminoácido de acuerdo a la secuencia de E6.

Tabla 2. Proteínas que interacción con E6 (Adaptada de Narisawa-Saito and Kiyono *et al.,* 2007, Mantovani *et al.,* 2001**)**

Proceso bológico	Proteína	Función celular	Efecto biológico de la interacción con E6	Referencias
*	E6AP/p53	Regula la transducción de señales en células en proliferación, induce apoptosis y es supresor de tumor	Desregulación de p53, inhibición de la apoptosis	Zur Hausen *et al*., 2002
Proteínas involucradas en adhesión célula-célula, polaridad y proliferación, mediante motivos de unión PDZ	MAGUK (hDlg 1, hDgl 4, hScrib)	Control de la polaridad y crecimiento celular Participa en la polaridad de células epiteliales en diferenciación.	Alteración de la adhesión celular, polaridad y proliferación.	Kiyono *et al.,* 1997
	MAGI-1/2/3	Participa en la traducción de señal. Forma un complejo con β-catenina.	Degradación de proteínas PDZ, pérdida de la polaridad celular	Glaunsinger *et al.,* 2000
	PTPN3	Participa en uniones entre la membranas y el citoesqueleto	Degradación de PTPN3	Jing *et al*.,2007
	CAL, Tip 1	Proteínas de señalización involucradas en la adhesión celular	Desregulación del tráfico vesicular, degradación de Cal y Tip vía proteosoma	Jeong *et al.,* 2007, Oliver *et al.,* 2011
	Mupp1	Control de la polaridad y crecimiento celular	Alteración del ensamblaje de los complejos de señalamiento en las membranas celulares.	Lee e*t al.,* 2000
Inmortalización	NFX1-91	Inhibidor de NFX1-91	Degradación de NFX1-9/activation of hTERT, inmortaliación	Gewin *et al.*, 2004
Apoptosis	Bak	Induce apoptosis	Degradación de Bak/ supresión de la apoptosis	Thomas *et al.,* 1999
	HIPK2	Gen antiapoptotico	Resistencia a la apoptosis	Muschik *et al.,* 2011

	c-MyC	Induce apoptosis.	Inhibición de la apoptosis.	Veldman *et al.,* 2003
	FADD	Activa la procaspasa-8	Degradación de FADD, inhibición de la apoptosis	Garnett *et al.,* 2006
	Procaspasa 8	Activa caspasas efectoras.	Degradación de procaspasa 8, supresión de la apoptosis	Garnett *et al.*, 2006
Respuesta inmune	IRF3	Factor transcripcional	Inhibición de la actividad transcripcional de IRF-3, inhibiendo la señalización inducida por IRF3	Ronco *et al.,* 1998
Proteínas incluidas en la regulación de transcripción y replicación del ADN	CBP/P300	Coactivador transcripcional	Desregulación en la actividad de p53 debido a que CBP/300 es el coactivador transcripcional de p53	Zimmermann *et al.,* 1999
	hMCM7	Inicio de la replicación del ADN	Induccion de anormalidades cromosomales	Kukimotto *et al.,* 1998
	E6TP1	Activa la GTPasa, proteina putativa GAP	Inhibición de la señal mitogénica mediada por Rab.	Gao *et al.,* 1999
	Gps2	Coactivador transcripcional	Degradación de GPS2	Degenhardt and Silverstein 2001
	Tuberina	Participa en vías de señalización de crecimiento	Crecimiento acelerado	Lu *et al.,* 2004
	XRCC1	Participa en la reparación del ADN.	IBloqueo de la reparación de ADN.	Iftner *et al.,* 2002
Proteínas involucradas con la organización epitelial y diferenciación	Paxilina	Traducción de señales. Ayuda a estabilizar el citoesqueleto de actina.	Interfiere en la asociación de paxiliana y la adhesión focal tipo quinasa	Mattiussi *et al.,* 2006
	E6BP/ERC-55	Proteina de inion a Ca^{2+}	Inhibición de la diferenciación terminal de células epiteliales.	Chen *et al.,* 1995

Se ha observado que Mupp1 interacciona con E6 del VPH 18 y HIPK2 interacciona con E6 del VPH 23

Tabla 3. Dominios de E6 del VPH 16 que interacciona con proteínas celulares (adaptada de Wise *et al.,* 2008, Pillai *et al.,* 2009)

Aminoácidos de E6 que participan en la unión con proteínas celulares	Proteína celular	Función de la proteína celular
△1-36,△ 95-131	*Gps2/AMF1	Inhibe la activación de JNK, es represor de la señal mediada por las MAPK y por la proteína Ras (de las siglas en inglés Mitogen-Activated Protein Kinases) (Degenhardt *et al*., 2001)
△ 1-91	$^{+}$hMCM7	Se une a RB e inhiben la replicación del DNA (Kukimoto *et al.,* 1998)
△ 100-147	p300/CBP2	Coactivadores transcripcionales de proteínas que participan en remodelación de la cromatina, crecimiento y diferenciación celular (Zimmermann *et al.,* 1999, Patel *et al.,* 1999)
△ 146-151	Proteínas con dominios de unión PDZ	Participa en la formación de uniones adherentes, adhesión célula-célula, polaridad y proliferación celular (Nguyen *et al.,* 2003, Mantovani *et al.,* 2001)
Cys-63 Arg/ Tyr 70 Cys/ Lys-72 Arg/ Thr-86 Ser, Cys-103 Arg/Asp- 120 Gly/ Ile-128 Met/Arg- 131 Pro, Gln- 90 Arg/Cys 111 Arg/Glu 113 stop	E6TP1	Presenta homología con RAP GAPs, y participa en proliferación y transformación celular (Gao *et al*., 1999, Gao *et al,* 2001)
$^{+}$C66G/C136G L50G	IRF3	Participa en inducción de la apoptosis (Park *et al.*, 2001)
L110Q, G130V, C 63R/Y70C/K72R/T86S	E6AP	El complejo E6-E6AP actúa como ubiquitin ligasa y degrada a la proteína que se une a este complejo (Liu *et al.,* 1999)
△ 118-122, L37, L110Q, W132R, C63R/Y70C/K72R/T86S	p53	Proteína supresora de tumor (Liu *et al.,* 1999)

R10G, Q14H, L19N, L19N, D25N, D25E, 126V, 127L, 127R, E29Q, E29K, R48W, F69L, S71C, Y76H, H78Y, H78C, L83V, L100S, H126Y, G130S, R135G, C140R , C140V, R141K, R141S	-	Epítope de celulas T (Pillai *et al.,* 2009)
R10G, Q14H, L19N, L100S, R135G, C140R , C140V, R141K, R141S, S142T, S142L, S143I	-	Epítope de células B (Pillai *et al.,* 2009)
R10G, Q14H, Y76H, H78Y, E113D, H126Y, G130S, R135G, C140R , C140V, R141K, R141S, R141S, S142T, S142L, S143I	-	Degradación y unión a p53 (Pillai *et al.,* 2009)
K34N	-	Dominios de unión a Zn (Pillai *et al.,* 2009)
F69L, H78Y, H78C, L83V, L100S, H126Y, R135G, R135G, C140R , C140V, R141K, R141S	-	Transactivación transcripcional de E6
E113D, H126Y, G130S	-	Unión de E6 a AP (Pillai *et al.,* 2009)
L83V	-	Degradación de p53 (Pillai *et al.,* 2009)

Se determinó que estos aminoácidos de E6 participaban en la interacción E6-proteina celular mediante la deleción de aminoácidos o la inserción de polimorfismos en la secuencia de E6. *El estudio se realizó en VPH 6, sin embargo, los aminoácidos 1-36 y 95-131 son altamente conservados entre los VPH cutáneos y los de mucosa. $^{+}$Las sustituciones C66G/C136G disrumpe a los dedos de Zinc. △ Se realizó deleción de aminoacidos del gen de E6. (-) No se conoce la proteína con la que E6 interacciona, debido a que los resultados son obtenidos de un estudio *in silio,* el cual solo determinó la función afectada al presentarse el polimorfismo en E6.

Los VPH que infectan queratinocitos de humanos pertenecen al género alfa papillomavirus, están asociados con el desarrollo de lesiones precancerosas y cáncer cervical (de villiers *et al.,* 2004). El VPH 16 es el más frecuente en cáncer cervical y adenocarcinoma, la infección por VPH 16 se relaciona con alto riesgo para desarrollar cáncer cervical (Ennaifer *et al.*, 2015, Molijn *et al.,* 2015). Existen variantes del VPH 16, debido a polimorfismos presentes en E6 y LCR del VPH 16, las variantes se agrupan en 4 linajes: Europea-Asiática (EAS), Africana 1 (AFR1), Africana 2 (AFR2), Norte Americana/Asiático Americana (NA/AA). Las variantes del VPH 16 presentan la siguiente nomenclatura: para las variantes Europeas, aparece la abreviación (E) de Europea, seguida de un guión, una letra y un número, para representar el nucleótido cambiado y la posición específica donde se llevó a cabo el cambio del nucleótido, por ejemplo: E-G350, es una variante Europea que presenta un polimorfismo en el nucleótido 350. El polimorfismo generó un cambio de nucleótido de una T por una G. Para los otros linajes se coloca la abreviación que representa al linaje seguido de una letra que representa al sublinaje (Tabla 4) (Huertas *et al.,* 2011, Cornet *et al.,* 2012)

Yamada *et al.,* (1997), determinaron que las variantes del VPH presentan una frecuencia en cáncer cervical relacionada al área geográfica de la cual eran procedentes las mujeres con cáncer cervical, las variantes Asiáticas son encontradas principalmente al sureste de Asia, las variantes Africanas son identificadas en tumores de mujeres africanas, en Europa y Norte América, las variantes Europeas son las que preodominan. A pesar de lo anterior, en otras poblaciones existe una heterogeneidad en cuanto a la asociación de infección por variantes del VPH 16 y la zona geográfica en la cual se encuentran las pacientes que presentan la infección. La distribución geográfica de las variantes de E6 del VPH 16 puede ser denotada por varios factores incluyendo, persistencia, migración de los individuos (Yamada et al., 1997) y el que las poblaciones se mezclen sexualmente, además de que factores genéticos pueden predisponer al establecimiento y/o persistencia de las infecciones por determinadas variantes de VPH 16 (Xi *et al*., 2006).

Tabla 4: Clasificación de las variantes del VPH 16 (Adaptado de Huertas-Salgado *et al.,* 2011, Cornet *et al.,* 2012).

Linaje	Sublinajes	Clase	Nucleotidos																				Aminoácido
		VPH 16 R	83 **A**	131 **A**	132 **G**	137 **T**	143 **C**	145 **G**	161 **C**	178 **T**	183 **T**	188 **G**	255 **G**	285 **C**	286 **T**	289 **A**	295 **T**	335 **C**	350 **T**	419 **T**	403 **A**	422 **A**	532 **A**	
Europea/ Asiática	Europea	E-Prototipo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		E-T350	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		E-G350	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	L83V
		E-A350	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	L83M
		As-b/r	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	D25E
		As-a/r	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	D25E
	Asiática	As-c/r	-	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Norte Americana/ Asiático Americana	Asiático Americana	AA-a/r	-	-	-	-	-	T	-	-	-	-	-	-	a	g	-	T	G	-	-	-	g	Q14H/H78Y/L83V
		AA-b/r	-	-	-	-	-	T	-	-	-	-	-	-	a	g	-	-	G	-	-	-	g	Q14H/L83V
		AA-c/r	-	-	-	-	-	T	-	-	G	-	-	-	a	g	-	T	G	-	-	-	g	Q14H/I27R/H78Y/L83V
	Norte Americana	NA 1-a	-	-	-	-	-	T	-	-	-	-	-	-	a	g	-	T	-	-	-	-	-	Q14H/H78Y
		NA 1-b/r	-	-	-	-	-	T	-	-	-	-	-	-	a	g	-	T	G	-	-	-	-	Q14H/H78Y/L83V
		NA 1-c	-	-	-	-	-	T	-	-	G	-	-	-	a	g	-	T	G	-	-	-	-	Q14H/I27R/H78Y/L83V
		NA 1-d	-	-	C	-	-	T	-	-	-	-	-	-	a	g	-	-	-	-	-	-	-	R10T/Q14H
Africana 1	Africana 1	Af 1-a/r	*C	-	C	-	G	T	-	-	-	-	-	-	a	g	-	T	-	-	-	-	-	R10T/Q14D/H78Y
		Af 1-b/r	-	-	C	-	G	T	-	-	-	-	-	-	a	g	-	T	-	-	-	-	-	R10T/Q14D/H78Y
		Af 1-c/r	ND	G	-	-	G	T	-	-	-	C	-	-	a	g	-	T	-	-	-	-	-	R10G/Q14D/E29Q/H78Y
		Af 1-d/r	ND	G	-	-	G	T	-	-	-	-	-	-	a	g	-	T	G	-	-	-	-	R10G/Q14D/H78Y/L83V
		Af 1-e/r	ND	-	-	-	G	T	G	-	-	-	T	-	a	g	G	T	G	-	-	-	-	Q14D/Q20E/C51F/D64E/H78Y / L83V
		Af 1-u/G419	*C	-	C	-	G	T	-	-	-	-	-	-	-	-	-	-	-	G	-	C	-	R10T/Q14D/C106C/E113D
Africana 2	Africana 2	Af 2-a/r	-	-	T	-	G	T	-	-	-	-	-	-	a	g	-	T	-	-	g	-	-	R10I/Q14D/H78Y
		Af 2-b/r	ND	-	-	-	G	T	-	-	-	-	-	-	a	g	-	T	-	-	-	-	-	Q14D/H78Y
		Af 2-c/r	ND	-	-	A	G	T	-	-	-	-	-	G	a	g	-	T	-	-	-	-	-	L12I/Q14D/A61G/H78Y

VPH16 R: Secuencia de referencia, las letras mayúsculas indican mutaciones que se traducen a cambos de aminoácidos, las letras minúsculas indican mutaciones silenciosas, las letras mayúsculas con un asterisco indican cambios de aminoácidos que no se consideran en la predicción de aminoácidos modificados, (-) indica que no hay mutaciones, ND: no determinado, las letras marcadas en gris indican mutuaciones caracteristicas para la rama filogenetica. La variante E-Prototipo no presenta cambios en comparación con la secuencia de E6 de referencia, E-T350 representa a una clase que no presenta cambios en el nucleótido 350, pero que presenta cambios en otros nucleótidos que codifican para E6. Los nucleótidos 143 y 145 codifican para el mismo aminoácido (aminoácido 14).
En la tabla aparecen los cambios de nucleótidos presentes en E6, sin embargo, Cornet *et al*., 2012 clasifica las variantes en base a E6 y LCR, en base a LCR la variante As-b pertenece a la clase europea y existen 2 clases de AA, AA1 y AA2 (datos no mostrados).

Las variantes de E6 del VPH 16 difieren en su potencial oncogénico dependiendo de la población (Xi *et al.,* 2007, Bhattacharjee *et al.,* 2008), esto está asociado con el fondo genético. El polimorfismo del oncogen E6 de la variante E-G350 puede interferir en la asociación entre el antígeno leucacitario humano (HLA) y cáncer cervical, en pacientes con polimorfismos haploides en HLA-DRB1*0401-DRB1*0301 and DRB*1101-DQB1*0301, la infección por VPH 16 de la variante E-G350 es más frecuente en comparación de cuando estos polimorfismos se encuentran ausentes (de Araujo *et al*.,2008).

Las variantes de E6 del VPH 16 tienen diferente potencial oncogénico, la expresión de E6 de las variantes del VPH 16 puede inducir la alteración en la expresión de genes celulares que participan en el desarrollo de cáncer cervical. Se ha observado que la expresión de genes a nivel de mRNA en células C33-A es alterada cuando existe expresión de E6 de la variante asiática (D25E), en comparación de cuando se expresa E6 de E-Prototipo, la expresión deE6 de la variante asiática des-regula nueve genes: ZMZ1, RPL23, MAPK4, RPL31, RARS, LAMB3, HSPA14, AIFM2, IFRD1 y sobre-regula cinco genes: UBC, RPS, HLA-A, -B, ROCK2, la modificación en la expresión de genes celulares favorece la oncogénesis de E6 de la variante D25E (Jang *et al.* 2011) debido a que los genes alterados participan en apoptosis, proliferación, ciclo celular, entre otros (Degenhardt *et al.,* 2011, Kukimoto *et al.,* 1998).

La expresión de E6 de la variante AA disminuye la expresión de las proteínas YWHAZ y PARK7, pero aumenta la expresión de RAB 30, TKT y FUBP1, en comparación con E6 de E-Prototipo, cabe destacar que RAB 30 es un oncogen de la familia Ras, lo que puede favorecer la oncogénesis de la variante AA; asimismo la variante AA es capaz de modificar la expresión de proteínas que participan en las vías metabólicas de los aminoácidos, ácidos tricarboxílicos y glicólisis (IDH1, IDH12, SET, PKM2, GAPDH, AKR1C y PGM2), sugiriendo que estos cambios participan en la producción del efecto de Warburg favoreciendo la inmortalización y transformación celular (Richard *et al.,* 2010).

A pesar de que la variante AA es considerada una de las variantes más oncogénicas (Richard *et al.,* 2010), tanto la variante E-G350 como la variante AA

son capaces de inducir la sub-expresión de $p16^{IBK4a}$ (ciclo celular) e Integrina A4 (adhesión) y sobre-expresan a MMP1 y 2 (invasión), S100A4 (invasión y ciclo celular), SERPINE1 (invasión), FOS (proliferación y diferenciación) y MDM2 (ciclo celular). La diferencia entre estas dos variantes es que la variante AA sobre-expresa a IL8 y la variante E-G350 induce la sub-expresión de MAP2K1, la vía de las MAP juega un papel fundamental en la oncogénesis debido a que modula la proliferación, diferenciación y ciclo celular, el aumento en MP2K1 podría tener un papel importante en la oncogénesis inducida por E6 de la variante E-G350 (Sichero *et al.,* 2012).

Los polimorfismos presentes en las variantes del VPH 16 le confieren diferente potencial oncogénico; las variantes de E6 más frecuentes en cáncer cervical modifican procesos oncogénicos para conducir al desarrollo de cáncer cervical. (Pillai *et al.,* 2009, Zehbe *et al.,* 2009, Sichero *et al.,* 2012).

La variante E-G350 tiene una mayor capacidad para inducir proliferación, inmortalizar queratinocitos, degradar Bax e inhibir la apoptosis en comparación con E-Prototipo (Pillai *et al.,* 2009, Lurchachaiwong *et al.,* 2009, Zehbe *et al.,* 2009, Sichero *et al.* 2012). Sin embargo, E-G350 presenta la misma capacidad para inhibir el arresto del ciclo celular y para disminuir los niveles de p53 que las variantes Af (R10/L83V) y AA (Q14H/H78Y/L83V) (Zehbe *et al.,* 2009, Sichero *et al.,* 2012).

La variante Europea (R10G/L83V) induce la desregulación de la diferenciación de queratinocitos significativamente más eficientemente que la E-Prototipo y que la AA (Q14H/H78Y/L83V), por otro lado, la variante E-G350 induce una mayor resistencia a la apoptosis en comparación con las variantes Europea (R10G/L83V) y AA (Q14H/H78Y/L83V) (Zehbe *et al.* 2009).

La variante AA induce que las células sean más alargadas, hiperplásicas y queratinizadas en comparación con la E-prototipo (Richard *et al.,* 2010). Se ha demostrado que la variante AA presenta mayor potencial metástasico, capacidad de inmortalización y transformación celular y que favorece la transcripción de

oncogenes E6/E7 en comparación con la E-prototipo (Niccoli *et al.,* 2012, Xi *et al.,* 2007, Bhattacharjee *et al.,* 2008)

Se ha observado que variantes Europeas poco frecuentes en cáncer cervical (**1.**R8Q, **2.**R10G y **3.**R48W) en comparación con la variante Europea más frecuente en cáncer cervical (E-G350) presentan la misma capacidad para inhibir el arresto del ciclo celular e inhibición de la elevación de p53 (inducido por actinomicina D), sin embargo, las tres variantes poco frecuentes estudiadas y la E-G350 presentan diferencias en su capacidad para desregular la estratificación/diferenciación de queratinocitos, inducción de apoptosis e hiperactivación de la vía Wnt (Zehbe *et al.,* 2011).

Para la población guerrerense las variantes más oncogénicas son AA-a, AA-c, E-G350, E-A176/G350, E-C188/G350 ya que con mayor frecuencia se encuentran en pacientes que presentan cáncer cervical, además las pacientes infectadas con estas variantes presentan alto riesgo para desarrollar cáncer cervical en comparación de pacientes infectadas con la E-Prototipo. Se han realizado estudios con las variantes AA-a y E-G350 para determinar su potencial oncogénico. Sin embargo, aun no esta clara la menera en como estas variantes del VPH 16 modula procesos celulares para el desarrollo de cáncer, por otro lado no se han realizado estudios para determinar cómo pueden modular la progresión a cáncer cervical las variantes AA-c, E-A176/G350 y E-C188/G350. Se ha sugerido que la expresión de E6 de algunas variantes del VPH 16 induce la alteración en la expresión de genes celulares, esto podría influir en el desarrollo a cáncer cervical debido a que los genes alterados participan en procesos relacionados a la carcinogénesis, por lo anterior, el objetivo de este trabajo fue evaluar si la expresión de variantes de E6 del VPH 16 (AA-a, AA-c, E-G350, E-A176/G350 y E-C188/G350) altera la expresión del transcriptoma humano.

Capítulo 1

Changes in global gene expression profiles induced by HPV 16 E6 oncoprotein variants in cervical carcinoma C33-A cells

Title page

Changes in global gene expression profiles induced by HPV 16 E6 oncoprotein variants in cervical carcinoma C33-A cells

Ana Elvira Zacapala-Gómez[a], Oscar Del Moral-Hernández[a], Nicolás Villegas-Sepúlveda[b], Alfredo Hidalgo-Miranda[c], Sandra Lorena Romero-Córdoba[c], Fredy Omar Beltrán-Anaya[c], Marco Antonio Leyva-Vázquez[a], Luz del Carmen Alarcón-Romero[d], and Berenice Illades-Aguiar[a]*

[a]Laboratorio de Biomedicina Molecular, Unidad Académica de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero, Chilpancingo, Gro., México.

[b]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN) México, D.F. México.

[c]Laboratorio de Genómica del Cáncer, Instituto Nacional de Medicina Genómica (INMEGEN), México, D.F, México.

[d]Laboratorio de Investigación en Citopatología e Histoquímica, Unidad Académica de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero, Chilpancingo, Gro., México.

*Corresponding author.

Postal address: Av. Lázaro Cárdenas S/N, Chilpancingo, Gro. México, 39090.

Tel.: +52 747 4710901.

E-mail address: b.illadesaguiar@gmail.com (B. Illades-Aguiar)

E-mail addresses:

AEZG: zak_ana@yahoo.com.mx

ODMH: odelmoralh@gmail.com

NVS: nvillega@cinvestav.mx

AHM: ahidalgo@inmegen.gob.mx

SLRC: sromero_cordoba@hotmail.com

FOBA: frebeltran@hotmail.com

MALV: leyvamarco13@gmail.com

LCAR: luzdelcarmen14@gmail.com

Abstract

We analyzed the effects of the expression of HPV 16 E6 oncoprotein variants (AA-a, AA-c, E-A176/G350, E-C188/G350, E-G350), and the E- Prototype in global gene expression profiles in an *in vitro* model. E6 gene was cloned into an expression vector fused to GFP and was transfected in C33-A cells. Affymetrix GeneChip Human Transcriptome Array 2.0 platform was used to analyze the expression of over 245,000 coding transcripts. We found that HPV16 E6 variants altered the expression of 387 different genes in comparison with E-Prototype. The altered genes are involved in cellular processes related to the development of cervical carcinoma, such as adhesion, angiogenesis, apoptosis, differentiation, cell cycle, proliferation, transcription and protein translation. Our results show that polymorphic changes in HPV16 E6 natural variants are sufficient to alter the overall gene expression profile in C33-A cells, explaining in part the observed differences in oncogenic potential of HPV16 variants.

Key words: HPV 16 variants, HPV 16 E6 oncoprotein variants, C33-A cells, global gene expression, cervical carcinoma.

Introduction

It has been widely shown that chronic infection by High Risk Human Papillomavirus (HR-HPV) is the main risk factor for developing cervical carcinoma (CC) and its precursor lesions (Münger et al., 2004). Genotype 16 of the HR-HPV is

the most common in cervical carcinoma worldwide (Crosbie et al., 2013; Obeidat et al., 2013), and its oncogenic potential is mainly related to the action of oncoproteins E6 and E7. These oncoproteins have the ability to bind several cellular proteins and interfere with fundamental processes which promote cervical carcinogenesis, such as apoptosis, cell differentiation, adhesion, cell cycle, and immune response (Wise-Draper et al., 2008; Narisawa-Saito et al, 2007; Pim et al., 2010; Moody et al., 2010).

Several studies have suggested that HPV 16 variants may contribute to cancer development (Xi et al., 2007). Genetic studies have shown natural amino acid variants of HPV16 that are classified according to the polymorphic changes in the E6 gene and the LCR region (Cornet et al., 2012). The reference HPV 16 genome was sequenced in 1985 (Seedorf et al., 1985) and many variants have been found since that time. HPV 16 variants have been classified into four major lineages (European-Asian, African 1, African 2 and Asian-American/North-American) and nine sublineages (Cornet et al., 2012). The presence of polymorphisms that generate amino acid changes in the E6 oncoprotein has been shown. T350G, a common polymorphism in European variants, causes a leucine to valine change (L83V); T178G, common in Asian variants, leads D25E; G145T, common in Asian American and North American variants, leads Q14H; C143G, common in African variants, leads Q14D (Huertas-Salgado et al., 2011). Polymorphisms frequency depends on the population studied (Xi et al., 2007; Xi et al., 2006; Cornet et al., 2012; Tornesello et al., 2011; Pillai et al., 2009).

For example, some studies of HPV16 variants in Mexico have shown that even in the same country its distribution is different depending on the region analyzed. Our laboratory in southern Mexico has recently reported that the risk of developing

cervical cancer in women infected with AA-a, AA-c, E-G350, E-C188/G350 and E-A176/G350 variants ranges from 69.01 (AA-a) to 10.48 fold (E-C188/G350), compared to women infected with HPV16 E-Prototype (reference HPV 16 sequence) (Ortiz-Ortiz et al., 2015).

Some studies have analyzed the effect of HPV16 E6 oncoprotein variants overexpression in primary cultures of keratinocytes and found that variants differ in their ability to induce serum/calcium-resistant colonies and down-regulation of p53 and Bax (Asadurian et al., 2007), affecting several important cellular processes, including differentiation, apoptosis (Zehbe et al., 2011; Zehbe et al., 2009), immortalization, transformation (Richard et al., 2010, Niccoli et al., 2012), migration and metastasis (Niccoli et al., 2012). Thus, experimental evidence supports the theory that intra-typical variations can affect the carcinogenic potential of HPV16, posed several years before (Bernard et al., 2006). Even though the effect of HPV16 E6 oncoprotein variants on processes related to carcinogenesis has been demonstrated, there are only a few studies that analyze their effect on the transcriptome at a global level. Jang et al. (2011) reported that the Asian variant (D25E) increases the expression of nine genes (ZMZ1, RPL23, MAPK4, RPL31, RARS, LAMB3, HSPA14, AIFM2, and IFRD1) and decreases the expression of five genes (UBC, RPS9, HLA-a, HLA-B, and ROCK2). Even if the specific functions of these genes in cervical oncogenesis remain to be elucidated, they conclude that E6 D25E might have a unique oncogenic role in cancerous transformation. In another study, a panel of 84 selected cellular genes involved in transformation and tumorigenesis was analyzed in primary human foreskin keratinocytes transduced with HPV 16 E6 and E7. They

found that E-Prototype, AA, and E-G350 variants significantly affected the expression of p16, MMP, MMP2, S100A4, SERPINE1, FOS, and MDM2. Furthermore, they found that upregulation of IL8 and MAP2K1 were specifically detected in AA and E-350G infected cells, respectively, independently of passages (Sichero et al., 2012).

To our knowledge, there are no studies that analyze the global gene expression profile at the transcriptional level induced by the HPV16 E6 oncoprotein variants. We have previously reported the frequency of HPV16 variants in southern Mexico and found that the most common variants are E-G350, followed the E-Prototype, E-C188/G350, AA-a, AA-c, and E-A176/350. Furthermore, the AA variants of HPV16 were 15-fold more prevalent than E-Prototype in cervical carcinoma. Therefore, the aim of our study was to analyze the effect of E6 oncoprotein expression from AA-a, AA-c, E-G350, E-A176/G350 and E-C188/G350 variants in global gene expression in an *in vitro* model in comparison with the E-Prototype, in order to provide a better understanding of the previously observed differences in their ability to promote immortalization, transformation, and migration. We found that HPV16 variants altered the expression of 387 different genes compared with the E-Prototype, and that there is a differential profile of genes altered by each HPV16 variant in C33-A cells. Interestingly, the altered genes are involved in cellular processes associated with the development of cervical cancer such as adhesion, angiogenesis, apoptosis, cell migration, tyrosine kinase signaling pathway, and proliferation.

Materials and methods

HPV16 E6 gene variants isolation from cervical samples

The DNA biobank of the Molecular Biomedicine Laboratory at the School of Chemistry and Biology of the Autonomous University of Guerrero in Chilpancingo, Guerrero, Mexico, was searched for cervical DNA samples with E-G350, E-A176/G350, E-C188/G350, AA-a and AA-c HPV16 E6 variants, and HPV16 E-Prototype. These samples came from cervical scrapings of women from southern Mexico that had given prior informed consent PCR was performed to amplify HPV16 E6 gene region (nucleotides 48 to 622) with primers HPV16-F048/HPV16-R622 (Casas et al., 1999). To confirm the identity of each variant, each PCR product was sequenced in automated ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences obtained were aligned with the reference sequence of HPV16 E6 gene (NC_001526.2) as we have previously reported (Ortiz-Ortiz et al., 2015).

Cloning HPV16 E6 variants into pEGFP-N1vector

To construct the pE6/EGFP-N1 vector, the sequence of HPV16 E6 gene variants AA-a, AA-c, E-A176/G350, E-C188/G350, E-G350, and E-Prototype was amplified by PCR using the primer pair E6-beginHindIII and E6-endBamHI (Del Moral-Hernández et al., 2010) to include restriction sites and eliminate the stop. All constructs were cloned in frame at the BamH1 site restriction of the pEGFP-N1 vector (Clontech Laboratories, Inc). The E6 carboxy terminus was connected to the GFP protein by a seven-amino acid peptide that resulted from the translation of the linker sequence (21 nt) between the *Bam*H1 site and the start codon of GFP (Del Moral-Hernández, et al. 2010). Constructs were verified by sequencing using a Big Dye

Terminator Ready Reaction Kit in the ABI PRISM 310 Genetic Analyzer System (Perkin-Elmer, Branchburg, NJ, USA).

Cell culture and stable transfection of C33-A cells

The cervical carcinoma cell line C33-A used in a previous report (Del Moral-Hernández, et al., 2010), was grown at 37 °C in 5% CO_2, using MEM supplemented with 10% fetal bovine serum (Gibco, Life Technologies, USA), 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were stably transfected using the standard calcium phosphate co-precipitation method (Jordan et al., 2004). Briefly, cells were grown for 24 h, transfected with 10 µg of each DNA construct and selected in MEM medium supplemented with 500 µg/ml G418 (Invitrogen, Corp.). After 4 weeks of selection, GFP-positive cells were sorted by FACS (FACS Calibur, BD, Mountain View, CA, USA) to obtain a purity of greater than 90%. The transfected cells were not cloned, but each experiment was repeated in at least three different independent assays.

Gene expression analysis

Total RNA was extracted from non-transfected C33-A cell cultures, stably transfected cell cultures and cells transfected with an empty vector, using Trizol (Invitrogen) and following the manufacturer's protocol. Equimolar concentrations of total RNA from 3 independent experiments were pooled, a total of 2 biological pools (n=3) per condition were made. The RNA quality was evaluated by capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies), only RNA samples

with an RNA Integrity Number greater than 8.0 were further processed for microarray analysis. 200 ng of RNA from each experimental condition were evaluated in the in the Gene Chip Human Transcriptome Array (HTA) 2.0 (Affymetrix) to define the whole transcriptome expression profiles according to the manufacturer´s protocol (See list of the differentially expressed genes in GEO ID: GSE73761). Briefly, the cDNA synthesis, amplification, and gene expression profiling were done with the WT PLUS Reagent Kit for fresh samples (Affymetrix, Santa Clara, USA). Wash and stain processes were performed with the Genechip hybridization wash and stain kit in the GeneChip Fluidics Station 450 (Affymetrix, Santa Clara CA, USA). The probe arrays were scanned using The GeneChip Scanner 3000 7G (Affymetrix, Santa Clara CA, USA). Array signal intensities were analyzed with the Affymetrix expression console. Briefly, raw data probes were normalized using robust multiarray analysis (RMA) for the background correction and quantile algorithm. To define the differential expression profiles within the different variants, a two-way Anova was performed in the Affymetrix Transcriptome Analysis Console (TAC) software. Genes with a fold change ≥ 1.5 or ≤ -1.5 and with a p-value ≤ 0.05 were considered significantly altered between the conditions. To remove the possible bias associated to the transfection procedure, we removed those genes that were significantly altered between non-transfected C33-A vs. C33-A transfected with the empty vector. To identify those biological processes altered by the E6 variants, we used DAVID software (http://david.abcc.ncifcrf.gov/summary.jsp), a bioinformatic tool for identification of the most relevant gene ontology process associated with the altered genes. Any process with an enrichment score greater than 0.5 was considered significant.

Real time RT-PCR

Total RNA was extracted from cultures of C33-A non-transfected cells, C33-A transfected with E6 from HPV16 variants, and C33-A transfected with empty vector, using Trizol (Invitrogen) following the manufacturer's instructions, then treated with DNase I, and finally reverse-transcribed using oligo-dT primers. cDNA synthesis was done as described according to the manufacturer's instructions with SuperScript® III Reverse Transcriptase enzyme (Invitrogen). To amplify the E6 transcripts the primer pair E6-F083 and E6R223 (Del Moral-Hernández, et al., 2010) was used. PCR was performed using 100 ng/µl of the cDNA and 0.5 µM of each primer. Real time PCR experiments were performed using the Power SYBRGreen PCR Master Mix and a Real Time ABI-PRISM 7500 SDS (Applied Biosystems). The amplification protocol was 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 60 s at 60 °C for annealing. We used the primer pair GAPDH-F0855/GAPDH-R1163 to amplify GAPDH (Truong et al., 2006) as endogenous control gene. Samples were analyzed in triplicate, and both negative and positive controls were included in each reaction.

For genes that were validated we used TaqMan (R) Gene Expression Master Mix (Applied Biosystems) probes [AMOTL1 (Hs00982921_m1), CDH2 (Hs00983061_m1), CDH6 (Hs01026788_m1), CDH9 (Hs00940349_m1), COL11A1 (Hs01097681_m1), NID1 (Hs00915876_m1), CALCR (Hs01016885_m1) and GAPDH (Hs99999905_m1). Each gene-specific cDNA was quantified in triplicate and mRNA ratios relative to the housekeeping gene GAPDH were calculated. The relative

quantification of mRNA was analyzed using method $2^{-\Delta\Delta Ct}$, and the means obtained from the triplicates are shown.

Western blot

Cells cultures with 80-95% confluence were treated with 10 μM proteasome inhibitor (Z-Leu-Leu-Leu-al) MG132 (Sigma-Aldrich) for 6 hours. Protein extraction was performed using the ProteoJET Cell™ Mammalian Cell Lysis Reagent (Thermo Scientific) kit following 2x PBS washes. Total protein (50 μg) was mixed with Laemmli sample buffer, boiled, separated by 12% SDS-PAGE, and transferred to a PVDF membrane (Perkin Elmer). Anti-GFP primary (Rockland) and secondary anti-goat-HRP antibodies were used (Santa Cruz). Anti-β-actin and anti-mouse-HRP were used as a loading control (donated by Dr. JM Hernández, CINVESTAV-IPN). Visualization was done on the MP ChemiDoc Imaging System (Bio Rad).

Results

Stable HPV16 E6 oncoprotein variants expression

The E6 oncoprotein gene of the HPV16 variants analyzed were cloned into the expression vector pEGFP-N1 to generate a fusion protein E6^GFP. C33-A cells were stably transfected and selected by sorting to have greater than 90% purity (data not shown). E6 mRNA was quantified by qRT-PCR to confirm the expression of the transcript. We found that the level of E6 mRNA is similar in all the analyzed groups, and despite the differences observed, no statistically significant difference in expression was found when compared with the E-prototype (Fig. 1a). We also

analyzed the expression of the E6 protein by Western blot using an anti-GFP antibody. As shown in Fig. 1b, cells expressing the E6 oncoprotein of the variant E-A176/G350 express more than all other variants despite having similar levels of messenger. The other variants showed slight differences in E6 oncoprotein expression.

HPV16 E6 oncoprotein variants differentially alter expression genes

To assess the global expression profile in C33-A cervical cancer cells stably transfected with E6 oncoprotein of five different variants of HPV16, total RNA was evaluated with the Human Transcriptome Array 2.0 platform (Affymetrix). We defined the global gene expression profile induced by the expression of the E6 oncoprotein variants focusing our attention on the coding transcripts present in the array (Fig. 2) The comparison of the specific gene expression profiles established by the variants reveals that the most dramatic changes were observed in the AA-c and E-G350 ones (229 and 132 respectively altered genes). The E6 oncoprotein expression of the E-Prototype vs C33-A wild type alters the expression of 266 genes; additionally, the comparison between the variants and E-Prototype overlaps with some common altered genes but with a different expression magnitude reflected in a different fold change (Fig. 2, supplementary Table S1). On the other hand, we found that 436 genes were regulated exclusively by HPV16 E6 variants of which 278 were upregulated and 158 were down regulated (Fig. 3). Of the 436 genes mentioned, 387 were unique genes altered by E6 variants and 49 of them are overlapped between variants (Supplementary Fig. S1, supplementary Table S2).

Genes altered by effect of HPV16 E6 variants expression are involved in cancer related processes

To classify differentially expressed genes according to their function, we performed an analysis using gene ontology. We found that the altered genes are involved in several processes related to cancer such as adhesion, angiogenesis, apoptosis, cell cycle, differentiation, migration, proliferation, and cell signaling. The cellular processes with the largest number of genes altered were cell signaling (20.3%), transcription (16.7%) and adhesion (14.6%) (Fig. 4). The genes were classified according to their cellular process and fold change in relation to the E-Prototype. We did not find a gene expression pattern characteristic of the phylogenetic branch variants (AA or European). However, we found some genes (ARID5B) that were upregulated by the expression of E6 from AA-c and E-G350 variants and IGF1R and ROR1 by E6 from AA-a and E-G350 variants. Furthermore, we also found genes such as EPHA5, CTNNA2, and SYNE1 that decrease its expression by the effect of the divergent E6 variants AA-c and E-G350 (supplementary Table S3).

Functional enrichment analysis

In order to highlight the most relevant biological processes associated with the expression profile established by each E6 variants we performed an enrichment analysis using the DAVID database, which allowed us to measure the relationships among the different annotation terms. The enrichment analysis showed that AA-c, E-C188/G350 and E-G350 variants were those that showed more enrichment in processes such as angiogenesis, cell junctions, migration, immune response, apoptosis,

cell division, proliferation, and activation of kinases; as well as in signaling pathways TGF-beta and cell junction/cadherin/WNT. Genes of TGF-beta signaling pathway like TGFBR2, BMPR1B, THBS1, and BMP5, were altered by AA-c variant while the genes EZR, PVRL3, CDH2, NID1, PCDH9, ROBO2 and PCDHB15 of cell junction/Cadherin/WNT signaling were altered only by E-G350 variant (Fig. 5, supplementary Table S4).

Validation of the microarray data

To validate the microarray data we selected a group of genes involved in cell adhesion (AMOTL1, CDH2, CDH6, CDH9, COL11A1, NID1, NRCAM, and CALCR), which were validated by qRT-PCR analysis. As shown in Table 1, the results for most of the genes analyzed were consistent with the microarray data. Among the analyzed genes, COL11A1, CALCR and NRCAM were the most up-regulated. Expression of CDH2 (E-G350), CDH6 (AA-c), CDH9 (AA-a), COL11A1 (AA-a), NRCAM (AA-a) and CALCR (AA-c) was observed by qRT-PCR when the oncoprotein HPV16 E6 variants were expressed in comparison to the E-Prototype.

Discussion

In this study, we used a global approach to analyze the effect of E6 HPV16 oncoprotein variants expression AA-a, AA-c, E-A176/G350, E-C188/G350, and E-G350 in comparison with the E-Prototype transfected in the C33-A HPV-negative cervical cancer cell line on the transcriptome. It has been reported that the C33-A is a cell line containing mutations in pRB and p53 genes, so they do not need the

carcinogenic effects of E6 and E7 of the HR-HPV oncoproteins (Scheffner et al., 1991). Therefore, this model allows the study of the effects of E6 oncogene in an established cell line.

The variants analyzed were isolated from HPV16-infected cervical cancer or precursor lesions samples of women from southern Mexico. Our results indicate that there is a specific gene expression profile of each variant, and that most of these genes are involved in oncogenic processes such as adhesion, proliferation, apoptosis, migration and cell projection. To our knowledge, this is the first study that compares the overall transcription profile in cervical cancer cells due to the expression of the E6 oncoprotein of five variants of HPV16 and E-Prototype. Our results provide evidence that could partially explain the different behavior regarding the oncogenic potential of each variant, as has previously been suggested.

Epidemiological studies suggest that variants of E6 HPV16 have different oncogenic potential (Mosmann et al., 2015; Qmichou et al., 2013; Tornesello et al., 2011; Xi et al., 2007; Fontecha et al., 2015), mainly based on the distribution and frequency in cervical cancer and precursor lesions. Moreover, some experimental studies show that natural intratypic variability in E6 gene variants of the HPV16 are sufficient to alter cellular functional activities induced by E6 such as resistance to serum/calcium differentiation, extension of the life span of primary human keratinocytes and reduction of the expression levels of p53 and Bax in human immortalized keratinocytes (Asadurian et al., 2007), apoptosis (Zehbe et al., 2011; Zehbe et al., 2009), and transformation and immortalization of human keratinocytes (Sichero et al., 2012). However, the mechanisms responsible for these alterations, as

well as genes involved in its regulation, have not been elucidated. Unlike the studies in primary human keratinocytes that only allow the study of cell transformation events, the C33-A model allows us to study the effects of E6 oncogene independent of p53 in an established cancer cell.

All variants tested in this study have an L83V amino acid change (at the carboxyl terminus domain of E6) compared to E6 from E-Prototype. Various *in vitro* studies have reported that this amino acid change gives E6 greater efficiency in the degradation of p53, Bax and binding to E6AP, higher ability to abrogate PHFKs differentiation induced by serum and calcium, but less efficient binding to hDlg (Asadurian et al., 2007; Stöppler et al., 1996; Lichtig et al., 2006), immortalization and transformation abilities (Richard et al., 2010) and regulation of tumorigenesis by the NOTCH and RAS signaling pathways (Chakrabarti et al., 2004). We have previously reported that the E-G350 (L83V) is the most common variant in our region, but it is not the most associated with the development of cervical carcinoma (Ortiz-Ortiz et al., 2015). Interestingly, the expression of the E6 oncoprotein from E-G350 (L83V) variant modulates the expression of more genes than other European variants and AA-a in C33-A cells (Fig. 2). The modulation of many genes may confer some advantages that relate to its high prevalence, however, the function of regulated genes is likely also important to confer oncogenic potential.

In addition to the L83V change, four of the five variants studied show other amino acid changes. In the branch AA, the AA-a variant has two more changes (Q14H /H78Y), while the AA-c variant has three (Q14H/I27R/H78Y) (Huertas-Salgado et al., 2011). It has recently been reported that AA E6 has enhanced abilities over E-

Prototype E6 in driving the infected epithelium toward tumorigenesis in a three-dimensional keratinocyte model (Jackson et al., 2014). Our results show that expression of the E6 oncoprotein of variant AA-c (Q14H/I27R/H78Y/L83V) modulates expression of more genes compared to the other variants (Fig. 2). However, E6 AA-a variant only modifies the expression of 22 genes, although the two variants differ only in one residue (I27R). Because the importance of this amino acid on HPV16 E6 oncogenicity has not been studied, we can only suggest that its presence regulates the number of genes compared to E6 from AA-a variant, but a possible mechanism is not currently available in the literature. This result was unexpected since AA-a variant shows the highest association with the development of cervical carcinoma in our population (Ortiz-Ortiz et al., 2015), but it is possible that the small number of genes altered by AA-a variant is enough to establish tumorigenic pathways.

The variants of the European branch E-A176/G350 that presents an extra amino acid change at the D25N residue, and E-C188/G350 that has a modification in the residue E29Q (both additional changes to L83V) compared to E-Prototype, also differ in their ability to alter gene expression profiles. Our results shown that the E6 oncoprotein of E-A176/G350 variant modulates the expression of few genes compared with the other variants. It is surprising that only one gene is altered by this E6 variant and E-Prototype, however it is hard to explain because there are no studies that analyze the role of D25N on E6 oncoprotein function. However, in a previous study we found that this European variant is the most closely related with the development of cervical cancer in our region (Ortiz-Ortiz et al., 2015). Interestingly, we observed that cells transfected with the E-A176/G350 variant expresses more protein than all others

variants despite having similar levels of messenger. Although, it is difficult to explain this phenomenon, we consider that post-transcriptional regulation mechanism, such as alternative splicing, could influence this behavior, however this fact could be a limitation of this study. It has been reported that mutations in a single nucleotide in the HPV 16 E6 gene are sufficient to alter the splicing pattern (Lopez-Urrutia, et al., 2012), and therefore may generate different amounts of messenger useful to be translated to E6. Currently our group is investigating this hypothesis.

Our analysis show that there is not an apparent pattern in terms of transcriptional regulation that identifies each phylogenetic branch (European and Asian-American) since the behavior in the genes and the number of modulated transcripts varies widely.

Our results identified a total of 436 altered genes by the expression of E6 variants. According to our gene ontology analysis, the cells expressing HPV E6 oncoprotein of E-C188/G350 variant mainly promotes the upregulation of cell proliferation and activation of kinase activity, while the cells expressing the E6 oncoprotein of AA-a, AA-c and E-G350 variants favor cell-cell adhesion, protein kinase activity, and tyrosine kinase signaling pathway (Figs. 4 and 5).

This differential behavior might be responsible for the biological role of the variants in cervical cancer. Previous reports have analyzed the functional activity of the AA and E-G350 E6 variants, indicating that both have more oncogenic potential compared to the E-Prototype (Sichero et al., 2012; Zehbe et al., 2009; Jackson et al., 2014). However, this is the first report describing the *in vitro* analysis of the effect of E6 oncoprotein expression from AA-a, AA-c, E-C188/G350, E-A176/G350 and E-

G350 variants in global gene expression profile in a cervical cancer cell line. Our results indicate that the additional amino acid changes in E6 variants could be sufficient to confer different oncogenic potential; this hypothesis can be validated with functional analysis in future studies. However we can not overlook the possibility that variations in other HPV proteins are likely involved in the oncogenic potential of each variant, because it has been shown that the E7 oncoprotein is necessary for cell transformation (Togtema et al., 2015).

Among the most important genes altered as a result of the expression of the E6 oncoprotein of variants is cadherin, which is involved in cellular interactions through the formation of intercellular connections or adherent junctions (Takeichi, 1990). Because metastasis begins with local disruption of cell-cell interaction, changes in the expression of cadherins play a critical role in the progression of tumors (Frixen et al., 1991; Vleminckx et al., 1991). We observed that the C33-A cells expressing the E6 oncoprotein of E-G350 variant increase the expression of cadherin 2 (responsible for transendothelial migration), E6 of AA-c increase cadherin 18 and 6, and E6 of AA-a increase cadherin 9 (Table 1 and 2). Aberrant expression of these genes could be a late effect of the E6 oncoprotein in tumor cells enabling it to advance in their degree of malignancy. Interestingly, cadherin 6 overexpression is associated with tumor growth and metastases in kidney cells (Shimazui et al., 2004). On the other hand, we found that factor receptor insulin-like growth 1 (IGF1R) is overexpressed by effect of E6 AA-a and E-G350 variants. Previous studies have shown a role for IGF1R in cellular radio-resistance in cervical carcinoma cell lines (Kaneko et al., 2007). Moreno-Acosta et al. (2012) found that in 34% of patients with HPV16-positive cervical cancer

overexpression of IGF1R occurs. The overexpression of IGF1R is a predictive marker for patients undergoing radio therapy because overexpression of this receptor confers 28.6 times greater risk of treatment failure.

It has been reported that high risk HPV E6 interacts with several cellular proteins such as transcription factors, ubiquitin ligases, signal transduction proteins, scaffolding proteins, calcium binding proteins, apoptosis inducers, among others (Mantovanii and Banks, 2001). Therefore, amino acid changes in HPV E6 variant proteins may alter the binding with its target proteins, resulting transcription pattern changes. On the other hand, we do not know if the binding with PDZ proteins could be affected, because the E6 carboxy terminus was linked to GFP protein, which could be considered a limitation of the study.

It is interesting that there are many differences between E6 HPV 16 variants and the E-Prototype and it is still a big question how these differences will lead us to understand the mechanisms involved.

Conclusion

In summary, our results provide evidence that polymorphisms in the HPV16 E6 gene that lead to amino acid changes in the protein are enough to modulate the transcription of specific genes in each variant. For the first time, the comparison of global gene expression profile modulated by the expression of E6 oncoprotein of the five HPV16 variants more frequent in our region is reported. Each variant appears to have its own molecular signature that may confer an advantage in some cell functions but may limit it in others. Therefore, future functional studies based on the profile of

genes altered for each variant are needed to analyze the mechanisms that are involved and to determine their significance in tumor biology.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We thank Raúl Bonilla-Moreno for his technical assistance. We thank Juan Francisco Leyva and Travis Ashworth for reviewing the English style of the article. This study was supported by grant from CONACYT, México.

References

Asadurian, Y., Kurilin, H., Lichtig, H., Jackman, A., Gonen, P., Tommasino, M., Zehbe, I., Sherman, L., 2007. Activities of human papillomavirus 16 E6 natural variants in human keratinocytes. J Med Virol. 79 (11), 1751-1760. doi: 10.1002/jmv.20978.

Bernard, H.U., Calleja-Macias I.E., Dunn S.T., 2006. Genome variation of human papillomavirus types: phylogenetic and medical implications. Int J Cancer. 118 (5), 1071-1076. doi: 10.1002/ijc.21655.

Casas, L., Galvan, S.C., Ordoñez, R.M., Lopez, N., Guido, M., Berumen, J., 1999. Asian-american variants of human papillomavirus type 16 have extensive mutations in the E2 gene and arehighly amplified in cervical carcinomas. Int J Cancer. 83(4), 449-455.

Chakrabarti, O., Veeraraghavalu, K., Tergaonkar, V., Liu, Y., Androphy, E.J., Stanley, M.A., Krishna, S., 2004. Human papillomavirus type 16 E6 amino acid 83 variants enhance E6-mediated MAPK signaling and differentially regulate tumorigenesis by notch signaling and oncogenic Ras. J Virol. 78 (11), 5934-5945. doi: 10.1128/JVI.78.11.5934–5945.2004.

Cornet, I., Gheit, T., Franceschi, S., Vignat, J., Burk, R.D., Sylla, B.S., Tommasino, M., Clifford, G.M., 2012. Human papillomavirus type 16 genetic variants: phylogeny and classification based on E6 and LCR. J Virol. 86 (12), 6855-6861. doi: 10.1128/JVI.00483-12.

Crosbie, E.J., Einstein, M.H., Franceschi, S., Kitchener, H. C., 2013. Human papillomavirus and cervical cancer. Lancet. 382 (9895), 889-899. doi: 10.1016/S0140-6736(13)60022-7.

Del Moral-Hernández, O., López-Urrutia, E., Bonilla-Moreno, R., Martínez-Salazar, M., Arechaga-Ocampo, E., Berumen, J., Villegas-Sepúlveda, N., 2010. The HPV-16 E7 oncoprotein is expressed mainly from the unspliced E6/E7 transcript in cervical carcinoma C33-Acells. Arch Virol. 155(12), 1959-1970. doi: 10.1007/s00705-010-0787-9.

Fontecha, N., Basaras, M., Arrese, E., Hernáez, S., Andía, D., Cisterna, R., 2015. Human papillomavirus 16 variants may be identified by E6 gene analysis. Intervirology. 58 (3), 143-148. doi: 10.1159/000381745.

Frixen, U.H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., Löchner, D., Birchmeier, W., 1991. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol. 113 (1), 173-185.

Huertas-Salgado, A., Martín-Gámez, D.C., Moreno, P., Murillo, R., Bravo, M.M., Villa, L., Molano, M., 2011. E6 molecular variants of human papillomavirus (HPV)

type 16: an updated and unified criterion for clustering and nomenclature. Virology. 410 (1), 201-215. doi: 10.1016/j.virol.2010.10.039.

Jackson, R., Togtema, M., Lambert, P.F., Zehbe, I., 2014. Tumourigenesis driven by the human papillomavirus type 16 Asian-American E6 variant in a three-dimensional keratinocyte model. PLoS One. 9 (7), e101540. doi: 10.1371/journal.pone.0101540.

Jang, M., Rhee, J.E., Jang, D.H., Kim, S.S., 2011. Gene expression profiles are altered in human papillomavirus-16 E6 D25E-expressing cell lines. Virol J. 8, 453. doi: 10.1186/1743-422X-8-453.

Jordan, M., Wurm, F., 2004. Transfection of adherent and suspended cells by calcium phosphate. Methods. 33 (2), 136-143. doi: 10.1016/j.ymeth.2003.11.011.

Kaneko, H., Yu, D., Miura, M., 2007. Overexpression of IGF-I receptor in HeLa cells enhances in vivo radioresponse. Biochem Biophys Res Commun. 363 (4), 937-941. doi: 10.1016/j.bbrc.2007.09.070.

Lichtig, H., Algrisi, M., Botzer, L.E., Abadi, T., Verbitzky, Y., Jackman, A., Tommasino, M., Zehbe, I., Sherman, L., 2006. HPV16 E6 natural variants exhibit different activities in functional assays relevant to the carcinogenic potential of E6. Virology. 350 (1), 216-227. doi: 10.1016/j.virol.2006.01.038.

López-Urrutia, E., Valdés, J., Bonilla-Moreno, R. Martínez-Salazar, M., Martínez-García, M., Berumen, J., Villegas-Sepúlveda, N. 2012. A few nucleotide polymorphisms are sufficient to recruit nuclear factors differentially to the intron 1 of HPV-16 intratypic variants. Virus Res. 166 (1-2), 43-53. doi: 10.1016/j.virusres.2012.02.026.

Mantovani, F., Banks, L., 2001. The human papillomavirus E6 protein and its contribution to malignant progression. Oncogene. 20 (54), 7874-7887.

Moody, C.A., Laimins, L.A., 2010. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer. 10 (8), 550-560. doi: 10.1038/nrc2886.

Moreno-Acosta, P., Gamboa, O., Sanchez de Gomez, M., Cendales, R., Diaz, G.D., Romero, A., Balart Serra, J., Conrado, Z., Levy, A., Chargari, C., Magné, N., 2012. IGF1R gene expression as a predictive marker of response to ionizing radiation for patients with locally advanced HPV16-positive cervical cancer. Anticancer Res. 32 (10), 4319-4325.

Mosmann, J.P., Monetti, M.S., Frutos, M.C., Kiguen, A.X., Venezuela, R.F., Cuffini, C.G., 2015. Mutation detection of E6 and LCR genes from HPV 16 associated with carcinogenesis. Asian Pac J Cancer Prev. 16 (3), 1151-1157. doi: 10.7314/APJCP.2015.16.3.1151.

Münger, K., Baldwin, A., Edwards, K.M., Hayakawa, H., Nguyen, C.L., Owens, M., Grace, M., Huh, K., 2004. Mechanisms of human papillomavirus-induced oncogenesis. J Virol. 78 (21), 11451-11460. doi: 10.1128/JVI.78.21.11451–11460.2004.

Narisawa-Saito, M., Kiyono, T., 2007. Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and E7 proteins. Cancer Sci. 98 (10), 1505-1511. doi: 10.1111/j.1349-7006.2007.00546.x.

Niccoli, S., Abraham, S., Richard, C., Zehbe, I., 2012. The Asian-American E6 variant protein of human papillomavirus 16 alone is sufficient to promoteimmortalization, transformation, and migration of primary human foreskin keratinocytes. J Virol. 86 (22), 12384-12396. doi: 10.1128/JVI.01512-12.

Obeidat, B., Matalka, I., Mohtaseb, A., Jaradat, S., Hayajneh, W., Khasawneh, R., Haddad H., Obeidat, F., 2013. Prevalence and distribution of high-risk human

papillomavirus genotypes in cervical carcinoma, low-grade, and high-grade squamous intraepithelial lesions in Jordanian women. Eur J Gynaecol Oncol. 34 (3), 257-260.

Ortiz-Ortiz, J., Alarcón-Romero, L del C., Jiménez-López, M.A., Garzón-Barrientos, V.H., Calleja-Macías, I., Barrera-Saldaña, H-A., Leyva-Vázquez, M.A., Illades-Aguiar, B., 2015. Association of human papillomavirus 16 E6 variants with cervical carcinoma and precursor lesions in women from Southern Mexico. Virol J. 12, 29. doi: 10.1186/s12985-015-0242-3.

Pillai, M.R., Hariharan, R., Babu, J.M., Lakshmi, S., Chiplunkar, S.V., Patkar M., Tongaonkar, H., Dinshaw, K., Jayshree, R.S., Reddy, B.K., Siddiqui, M., Roychoudury, S., Saha, B., Abraham, P., Gnanamony, M., Peedicayil, A., Subhashini, J., Ram, T.S., Dey, B., Sharma, C., Jain, S.K., Singh, N., 2009. Molecular variants of HPV-16 associated with cervical cancer in Indian population. Int J Cancer. 125 (1), 91-103. doi: 10.1002/ijc.24322.

Pim, D., Banks, L., 2010. Interaction of viral oncoproteins with cellular target molecules: infection with high-risk vs low-risk human papillomaviruses. APMIS. 118 (6-7), 471-493. doi: 10.1111/j.1600-0463.2010.02618.x.

Qmichou, Z., Khyatti, M., Berraho, M., Ennaji, M.M., Benbacer, L., Nejjari, C., Benjaafar, N., Benider, A., Attaleb, M., El Mzibri, M., 2013. Analysis of mutations in the E6 oncogene of human papillomavirus 16 in cervical cancer isolates from Moroccan women. BMC Infect Dis. 13, 378. doi: 10.1186/1471-2334-13-378.

Richard, C., Lanner, C., Naryzhny, S.N., Sherman, L., Lee, H., Lambert, P.F., Zehbe, I., 2010. The immortalizing and transforming ability of two common human papillomavirus 16 E6 variants with different prevalences in cervical cancer. Oncogene. 29 (23), 3435-3445. doi: 10.1038/onc.2010.93.

Scheffner, M., Münger, K., Byrne, J.C., Howley, P.M., 1991. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc Natl Acad Sci U S A. 88 (13), 5523-5527.

Seedorf, K., Krämmer, G., Dürst, M., Suhai, S., Röwekamp, W.G., 1985. Human papillomavirus type 16 DNA sequence. Virology. 145(1), 181-185.

Shimazui, T., Yoshikawa, K., Uemura, H., Hirao, Y., Saga, S., Akaza, H., 2004. The level of cadherin-6 mRNA in peripheral blood is associated with the site of metastasis and with the subsequent occurrence of metastases in renal cell carcinoma. Cancer. 101 (5), 963-968. doi: 10.1002/cncr.20479.

Sichero, L., Sobrinho, J.S., Villa, L.L., 2012. Oncogenic potential diverge among human papillomavirus type 16 natural variants. Virology. 432 (1), 127-132. doi: 10.1016/j.virol.2012.06.011.

Stöppler, M.C., Ching, K., Stöppler, H., Clancy, K., Schlegel, R., Icenogle, J., 1996. Natural variants of the human papillomavirus type 16 E6 protein differ in their abilities to alter keratinocyte differentiation and to induce p53 degradation. J Virol. 70 (10), 6987-6993.

Takeichi, M., 1990. Cadherins: a molecular family important in selective cell-cell adhesion. Annu Rev Biochem. 59, 237-252. doi: 10.1146/annurev.bi.59.070190.001321.

Togtema, M., Jackson, R., Richard, C., Niccoli, S., Zehbe, I., 2015. The human papillomavirus 16 European-T350G E6 variant can immortalize but not transform keratinocytes in the absence of E7. Virology. 485, 274-282. doi: 10.1016/j.virol.2015.07.025.

Tornesello, M.L., Losito, S., Benincasa, G., Fulciniti, F., Botti, G., Greggi, S., Buonaguro, L., Buonaguro, F.M., 2011. Human papillomavirus (HPV) genotypes and HPV16 variants and risk of adenocarcinoma and squamous cell carcinoma of the cervix. Gynecol Oncol. 121 (1), 32-42. doi: 10.1016/j.ygyno.2010.12.005.

Truong, A.B., Kretz, M., Ridky, T.W., Kimmel, R., Khavari, P.A., 2006. p63 regulates proliferation and differentiation of developmentally mature keratinocytes. Genes Dev. 20(22), 3185-3197. doi: 10.1101/gad.1463206.

Vleminckx, K., Vakaet, L. Jr., Mareel, M., Fiers, W., van Roy, F., 1991. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell. 66 (1), 107-119. doi: 10.1016/0092-8674(91)90143-M.

Wise-Draper, T.M., Wells, S.I., 2008. Papillomavirus E6 and E7 proteins and their cellular targets. Front Biosci. 13,1003-1017. doi: 10.2741/2739.

Xi, L.F., Kiviat, N.B., Hildesheim, A., Galloway, D.A., Wheeler, C.M., Ho, J., Koutsky, L.A., 2006. Human papillomavirus type 16 and 18 variants: race-related distribution and persistence. J Natl Cancer Inst. 98 (15), 1045-1052. doi: 10.1093/jnci/djj297.

Xi, L.F., Koutsky, L.A., Hildesheim, A., Galloway, D.A., Wheeler, C.M., Winer, R.L., Ho, J., Kiviat, N,B., 2007. Risk for high-grade cervical intraepithelial neoplasia associated with variants of human papillomavirus types 16 and 18. Cancer Epidemiol Biomarkers Prev. 16 (1), 4-10. doi: 10.1158/1055-9965.EPI-06-0670.

Zehbe, I., Richard, C., DeCarlo, C.A., Shai, A., Lambert, P.F., Lichtig. H., Tommasino, M., Sherman, L., 2009. Human papillomavirus 16 E6 variants differ in their dysregulation of human keratinocyte differentiation and apoptosis. Virology. 383 (1), 69-77. doi: 10.1016/j.virol.2008.09.036.

Zehbe, I., Lichtig, H., Westerback, A., Lambert, P.F., Tommasino. M., Sherman, L., 2011. Rare human papillomavirus 16 E6 variants reveal significant oncogenic potential. Mol Cancer. 10, 77. doi: 10.1186/1476-4598-10-77.

Figure captions

Fig. 1. Expression of HPV16 E6 variants in C33-A cells. a) Relative expression of E6 mRNA from HPV 16 variants compared to E-Prototype in C33-A cells (determined by real-time PCR). Despite the differences observed, no statistically significant difference (Student's t-test) was found in the expression of E6. b) Expression level of E6 protein of HPV16 variants in C33-A cells analyzed by Western blot using anti-GFP antibody. Increased expression of E6 with variant E-A176/G350 is observed.

Fig. 2. HPV16 E6 oncoprotein variants differentially alter expression of genes in C33-A cells vs. E-Prototype. The Venn diagrams show unique and common differentially expressed genes (a fold change ≥ 1.5 and ≤ -1.5) between cells expressing the E6 oncoprotein of E–Prototype and E-6 variants. The p-value was calculated with FDR, p-value cut off of <0.05 were used.

Fig. 3. Global expression profile induced by the HPV16 E6 oncoprotein variants expression vs. E-Prototype. E6 oncoproteins from HPV16 variants differ in their capacity to upregulate and downregulate genes in C33-A cells. The AA-c and E-G350 variants had the most genes altered (111 vs. 118 and 109 vs. 23 respectively).

Fig. 4. Gene ontology-based biological process pathways related to cancer. The genes that were differentially expressed by E6 of HPV16 variants were grouped according to their function in biological processes for the development and maintenance of cancer. Cellular processes more affected were cell signaling and adhesion. Both upregulated and downregulated genes are shown. For the analysis were not included the overlapping genes between E-Prototype and HPV 16 variants.

Fig. 5. Enrichment score of biological processes modulated by HPV16 E6 variants. Pathway enrichment analysis reveals over-representation or under-representation of oncogenic pathways for the HPV variants based in the differentially expressed genes. The plot shows enrichment scores and percentage of involved genes according to the enrichment cluster analysis from DAVID. Upregulated (red), downregulated (blue).

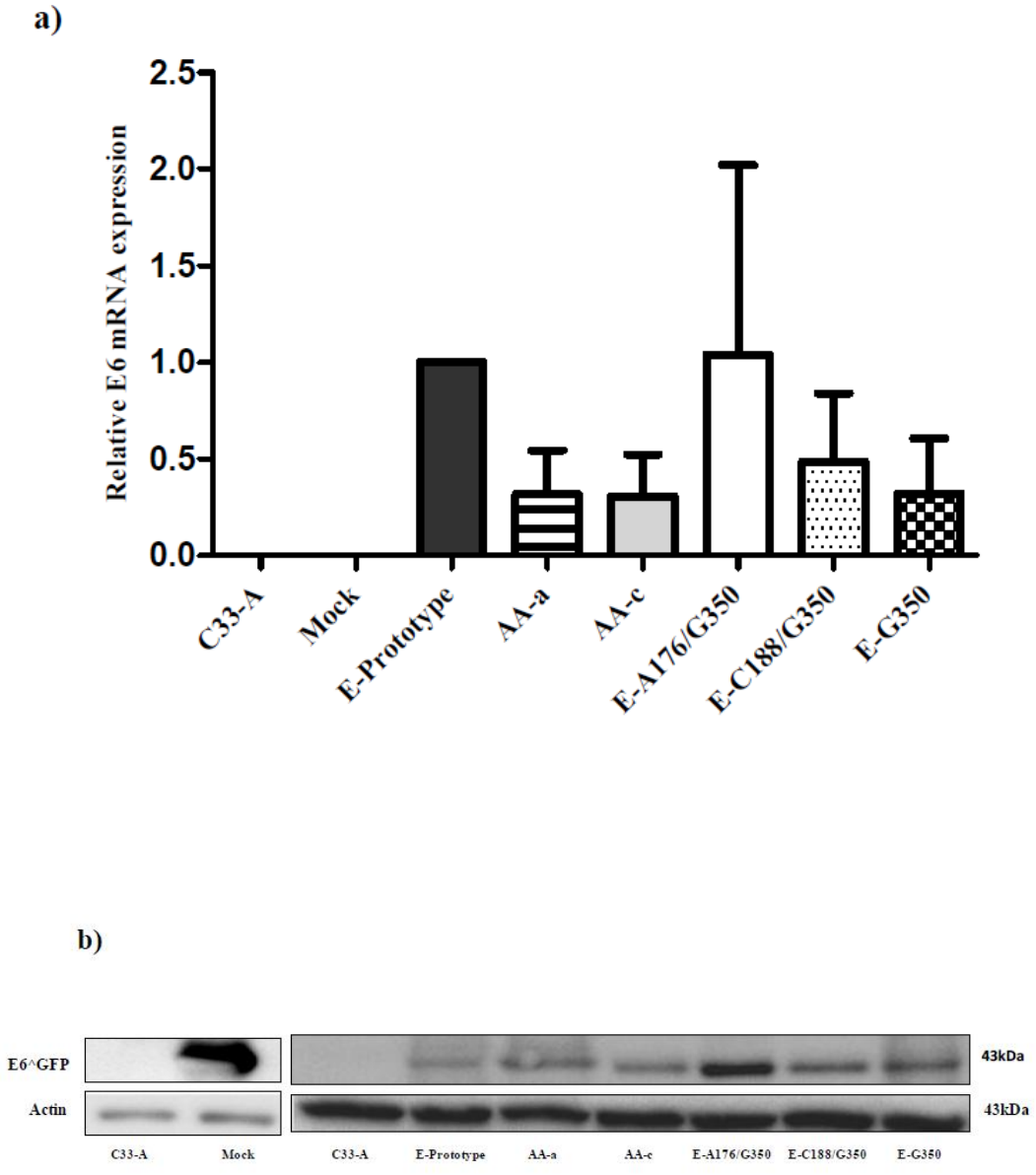

a)
Relative E6 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
C33-A
Mock
E-Prototype
AA-a
AA-c
E-A176/G350
E-C188/G350
E-G350
b)
E6^GFP
Actin
43kDa
43kDa
C33-A
Mock
C33-A
E-Prototype
AA-a
AA-c
E-A176/G350
E-C188/G350
E-G350

Figure 1

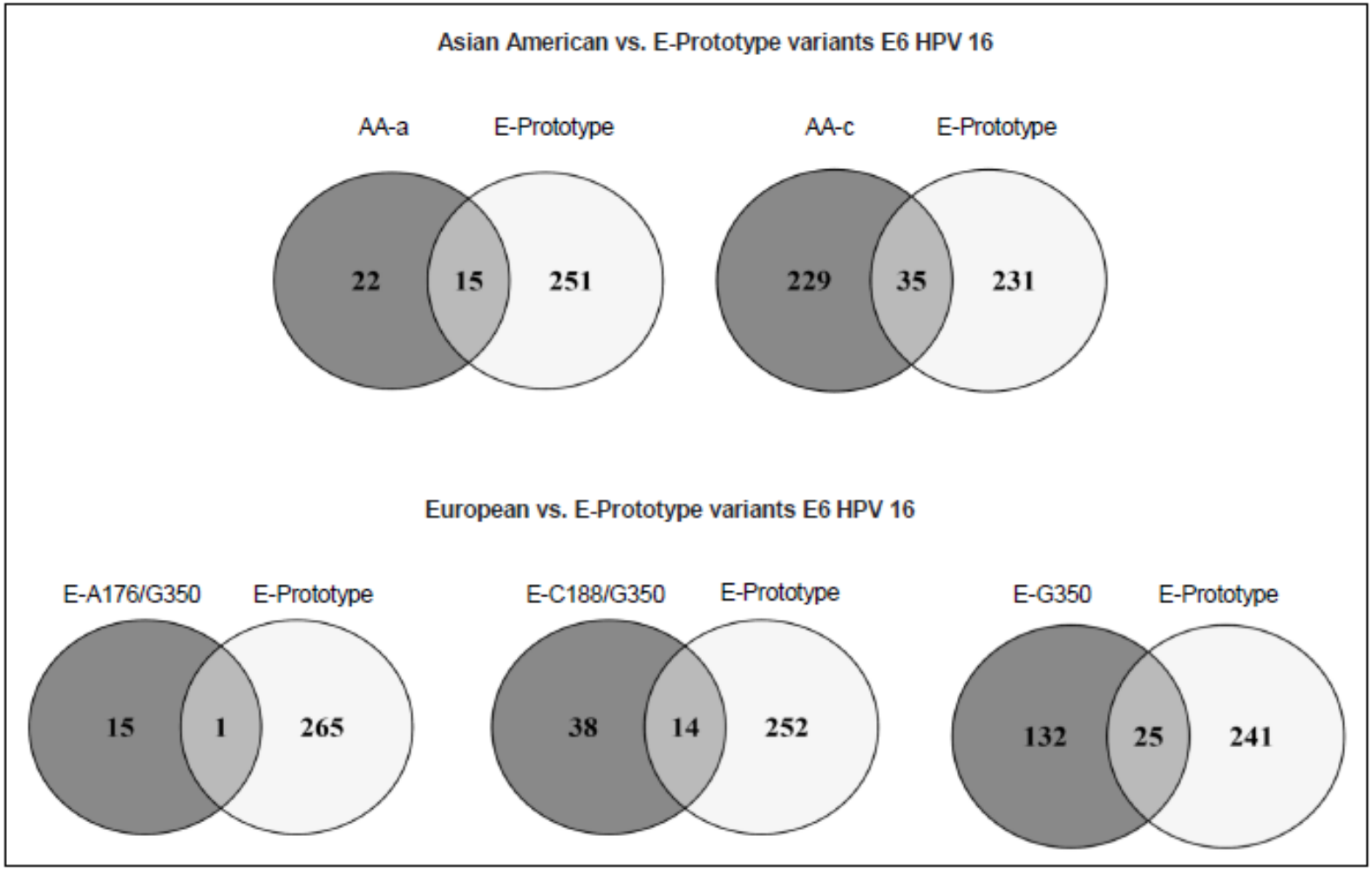

Asian American vs. E-Prototype variants E6 HPV 16
AA-a
E-Prototype
22
15
251
AA-c
E-Prototype
229
35
231
European vs. E-Prototype variants E6 HPV 16
E-A176/G350
E-Prototype
15
1
265
E-C188/G350
E-Prototype
38
14
252
E-G350
E-Prototype
132
25
241

Figure 2

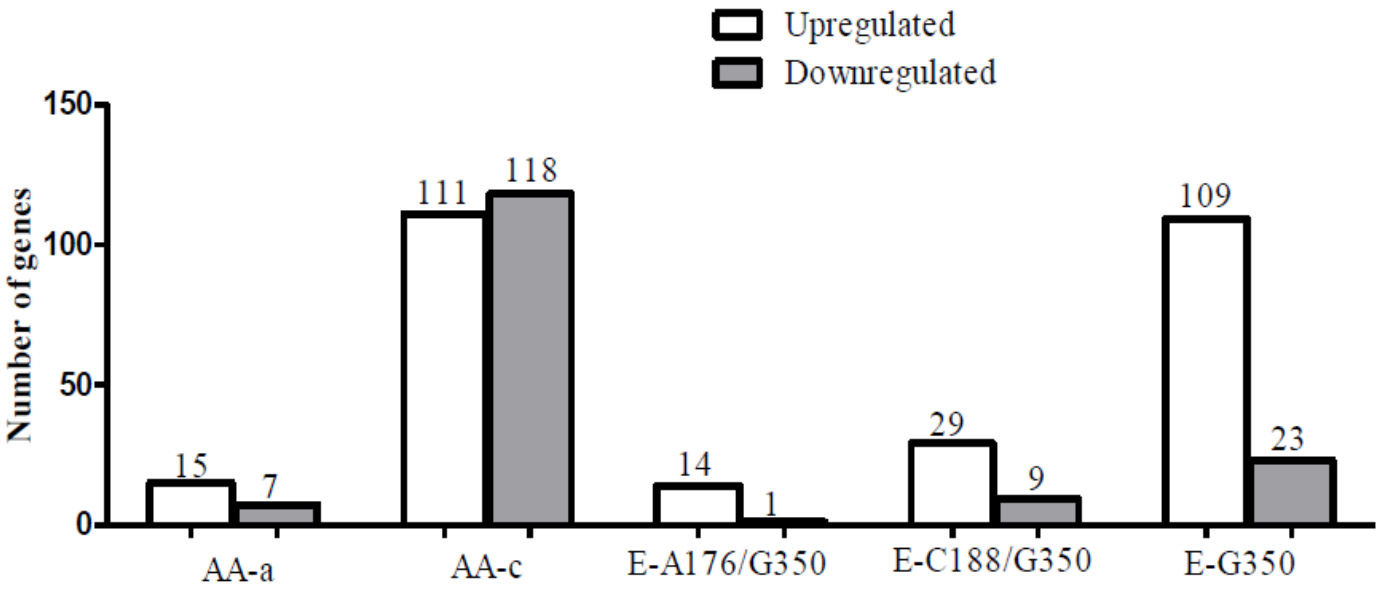

Upregulated
Downregulated
Number of genes
150
100
50
0
15
7
111
118
14
1
29
9
109
23
AA-a
AA-c
E-A176/G350
E-C188/G350
E-G350

Figure 3

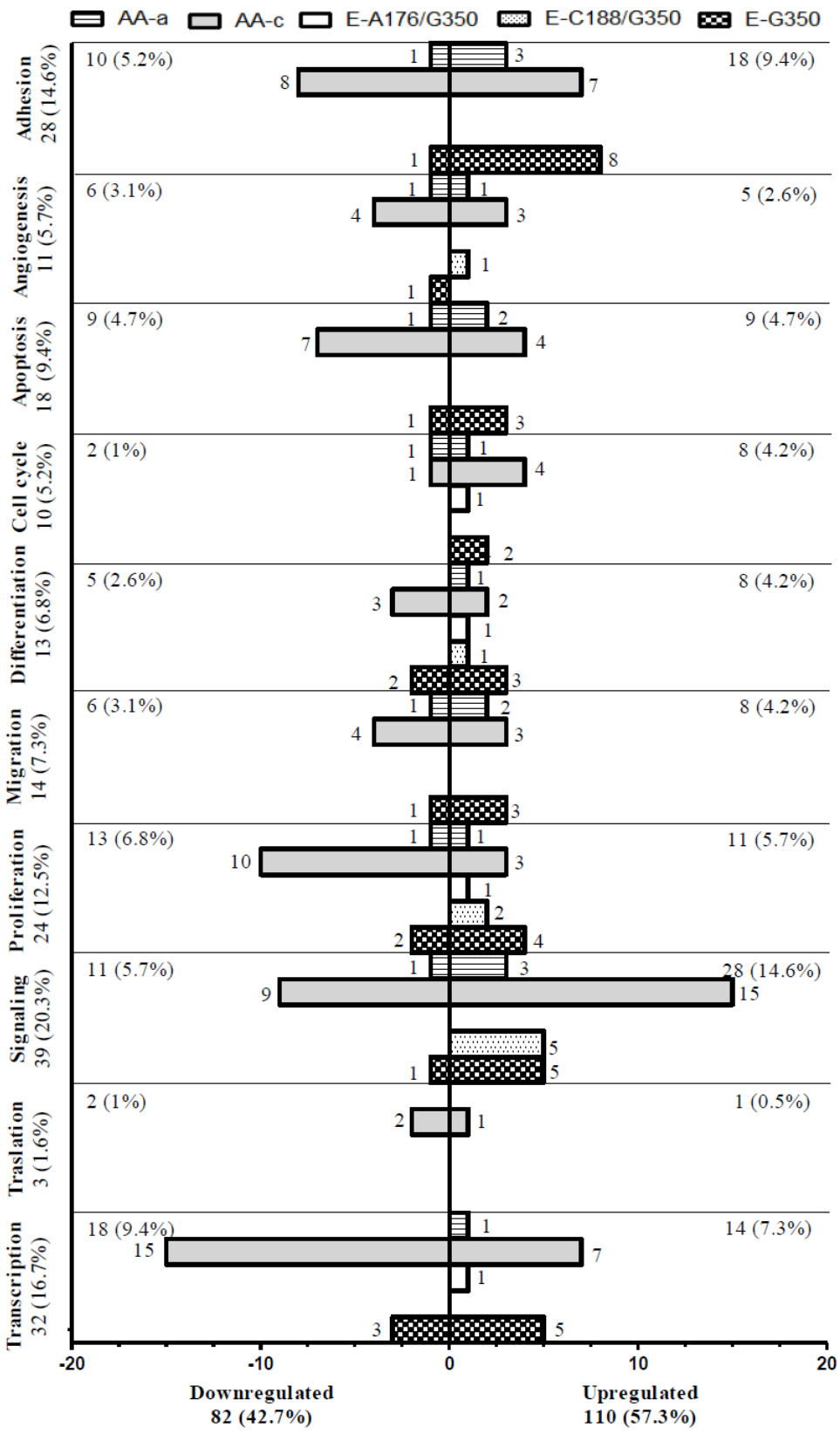

AA-a
AA-c
E-A176/G350
E-C188/G350
E-G350
Adhesion 28 (14.6%)
Angiogenesis 11 (5.7%)
Apoptosis 18 (9.4%)
Cell cycle 10 (5.2%)
Differentiation 13 (6.8%)
Migration 14 (7.3%)
Proliferation 24 (12.5%)
Signaling 39 (20.3%)
Traslation 3 (1.6%)
Transcription 32 (16.7%)
10 (5.2%)
18 (9.4%)
6 (3.1%)
5 (2.6%)
9 (4.7%)
9 (4.7%)
2 (1%)
8 (4.2%)
5 (2.6%)
8 (4.2%)
6 (3.1%)
8 (4.2%)
13 (6.8%)
11 (5.7%)
11 (5.7%)
28 (14.6%)
2 (1%)
1 (0.5%)
18 (9.4%)
14 (7.3%)
-20
-10
0
10
20
Downregulated
82 (42.7%)
Upregulated
110 (57.3%)

Figure 4

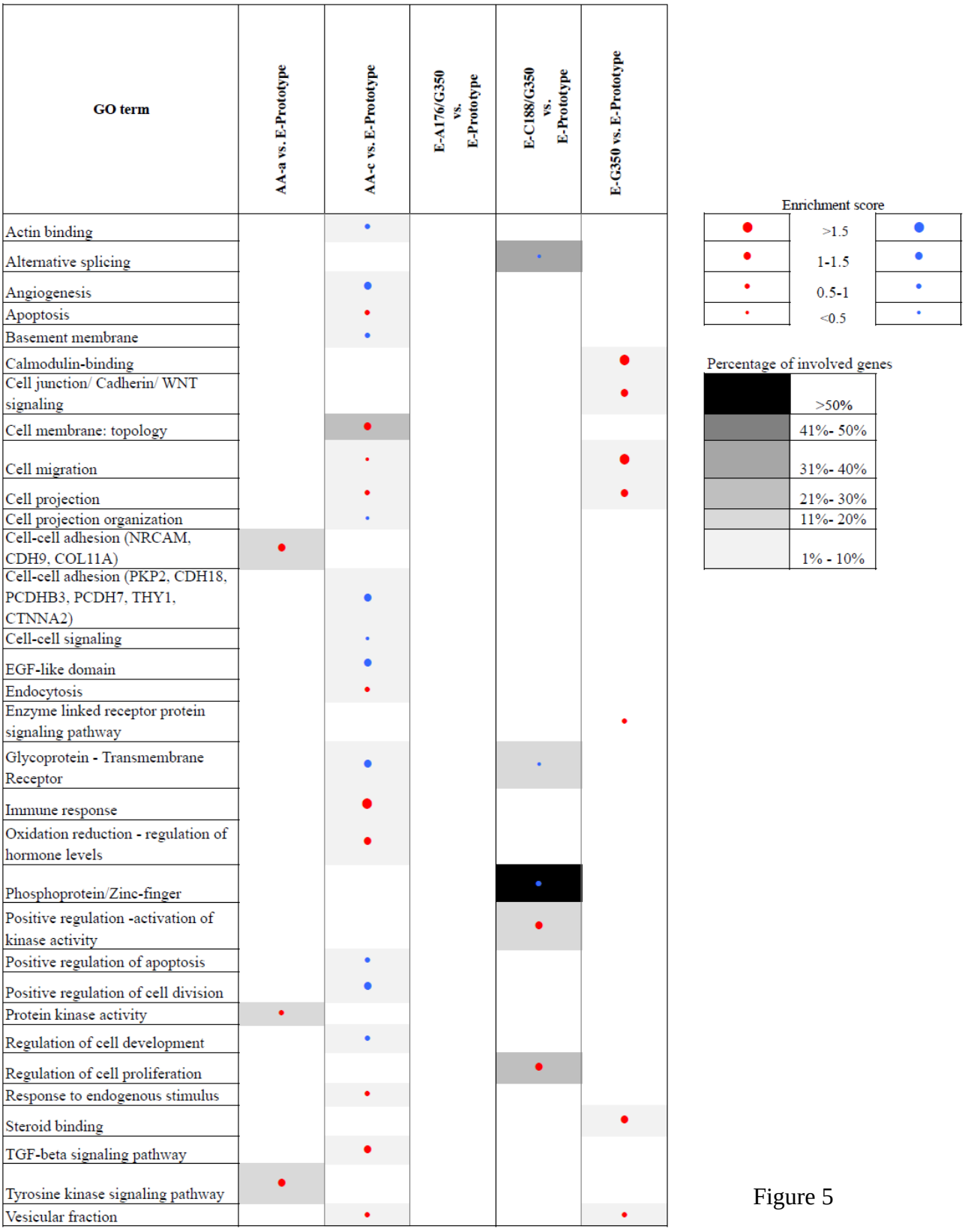

GO term
AA-a vs. E-Prototype
AA-c vs. E-Prototype
E-A176/G350 vs. E-Prototype
E-C188/G350 vs. E-Prototype
E-G350 vs. E-Prototype
Actin binding
Alternative splicing
Angiogenesis
Apoptosis
Basement membrane
Calmodulin-binding
Cell junction/ Cadherin/ WNT signaling
Cell membrane: topology
Cell migration
Cell projection
Cell projection organization
Cell-cell adhesion (NRCAM, CDH9, COL11A)
Cell-cell adhesion (PKP2, CDH18, PCDHB3, PCDH7, THY1, CTNNA2)
Cell-cell signaling
EGF-like domain
Endocytosis
Enzyme linked receptor protein signaling pathway
Glycoprotein - Transmembrane Receptor
Immune response
Oxidation reduction - regulation of hormone levels
Phosphoprotein/Zinc-finger
Positive regulation -activation of kinase activity
Positive regulation of apoptosis
Positive regulation of cell division
Protein kinase activity
Regulation of cell development
Regulation of cell proliferation
Response to endogenous stimulus
Steroid binding
TGF-beta signaling pathway
Tyrosine kinase signaling pathway
Vesicular fraction
Enrichment score
>1.5
1-1.5
0.5-1
<0.5
Percentage of involved genes
>50%
41%- 50%
31%- 40%
21%- 30%
11%- 20%
1% - 10%

Figure 5

Table 1. Validation on microarray data by RT-qPCR

	Fold change[b] determined by :					
	Microarrays			**qPCR**		
Gene[a]	AA-a	AA-c	E-G350	AA-a	AA-c	E-G350
AMOTL1	-	1.6	-	-	2.72	-
CDH2	-	-	1.65	-	-	2.35
CDH6	-	1.69	-	-	2.95	-
CDH9	1.9	-	-	5.97	-	-
COL11A1	6.01	-	-	19.86	-	-
NID1	-	-2.28	7.6	-	-	12.62
CALCR	-	61.23	-	-	120.88	-

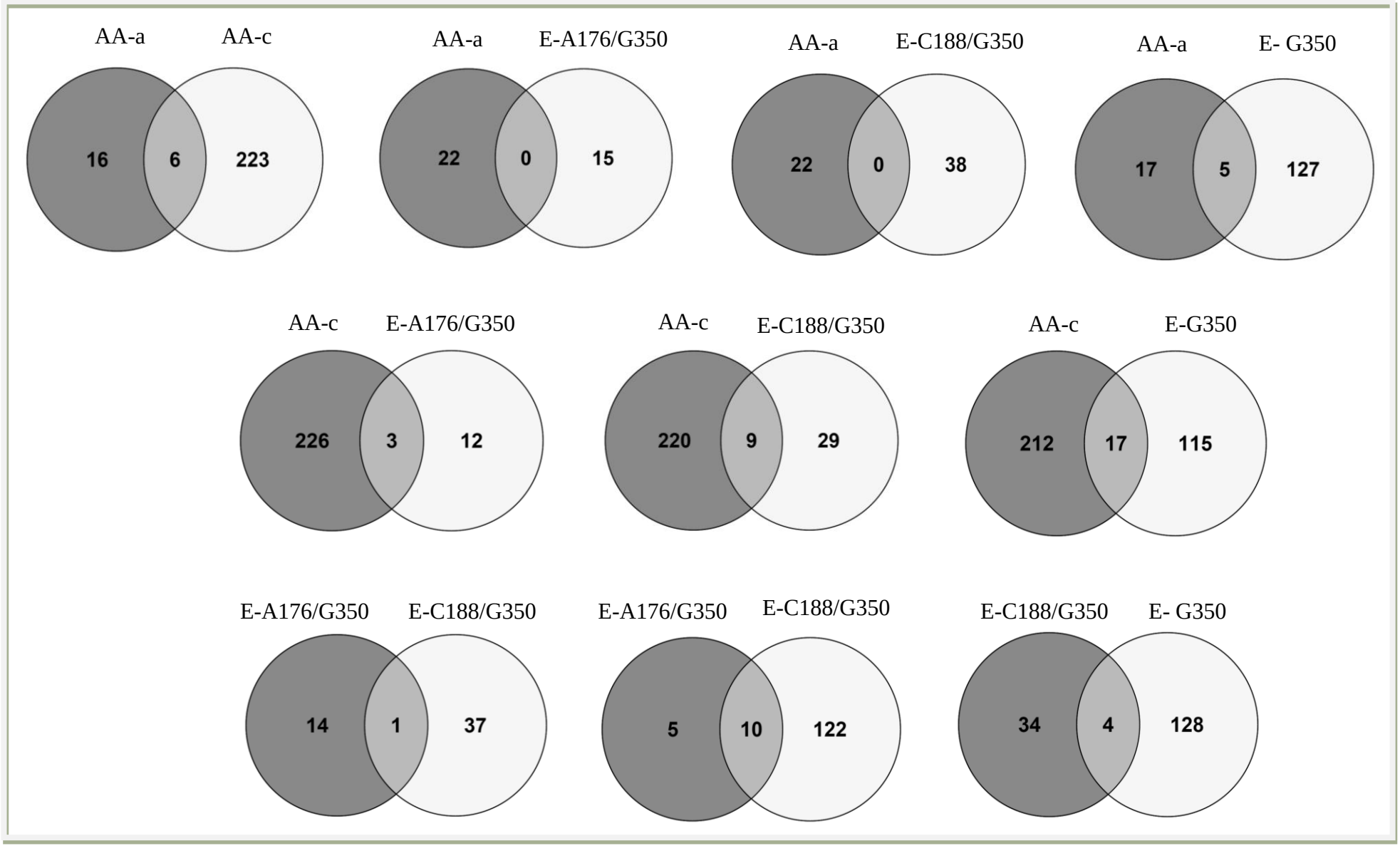
[a]**AMOTL1**: Angiomotin Like 1, **CDH2**: Cadherin 2, **CDH6**: Cadherin 6, **CDH9**: Cadherin 9, **COL11A1:** Collagen, Type XI, Alpha 1, **NID1**: Nidogen-1, **CALCR**: Calcitonin Receptor. The full names of the genes were taken from the GeneCards data base . (-) no alteration in the expression when compared to HPV E6 E-prototype.
[b] The values shown are means of three independent experiments.
All comparisons were done between E-Prototype vs. E-6 variants.

Supplementary Table S1. Genes modulated by both HPV16 E6 oncoprotein variants and E6 E-Prototype

Gene	Cellular process	Fold change E-Prototype	Fold change AA-a	Fold change AA-c	Fold change E-A176/ G350	Fold change E-C188/ G350	Fold change E-G350
AC007091.1	-	1.55	-	-1.7	-	-	-
AC133680.1	-	4.93	-4.26	-4.88	-	-3.92	-4.47
AKR1C2	N/F	-1.54	-	-	-	-	1.69
ALDH1A1	N/F	2.69	4.78	1.88	-	4.81	2.84
ARL9	N/F	1.57	-	-	-	-	-1.64
BCO2	N/F	2.29	-	1.97	-	-	-2.16
BMP5	Growth	-2.54	-	7.97	-	-	-
CEP170P1	-	1.54	-	-1.89	-	-	-
CFH	Immune response	4.95	-	-	-	-	-6.34
CUBN	Endocytosis	6.44	-7.24	-8.79	-	-6.06	-5.87
DCC	Apoptosis and cell motility	6.76	-	-9.76	-	-	-10.93
DSE	N/F	-1.52	-	1.56	-	-	-
EEA1	Endocytosis	2.34	-	-	-	-	-1.95
FMN2	Cytoskeleton organization and cell cycle	7.93	-10.95	-12.85	-	-8.03	-6.25
GAGE10	N/F	2.5	-	3.88	-	-	-
GAGE12C	Cellular defense	9.27	-	4.16	-	-	-
GAGE12J	N/F	7.05	-	4.79	-	-	-
HIST1H2AE	Packing of DNA	2.26	-	-	-	-	1.53
IFI30	Proliferation, post-transcriptional regulation, antigen processing and presentation	1.58	-	-2.01	-	-	-
KCNH7	Transcription	1.84	3.97	15.38	-	-	3.16
LPPR1	-	2.48	-2.67	-2.68		-2.56	-2.53
MAGEA1	-	-1.51	-	-	-	-	1.97

MARK1	Cell signaling and cytoskeletal organization	-1.66	1.54	1.69	-	-	-
MID1	Cytoskeleton organization	1.55	-1.77	-2.58	-	-	-
MUSK	Regulation of transcription , cell signaling and growth	1.72	-1.79	-1.76	-	-1.82	-
NPFFR2	Cell signaling	2.28	-	-2.64	-	-2.13	-
PCDH7	Adherence	2.93	-	-3.58	-	-	-
PLCB4	Cell signaling	1.95	-	-1.81	-	5.69	-
PPP3CA	Cell cycle	-2.05	-	2.29	-	-	1.62
PREX2	Cell signaling	1.88	-	-	-	-	-1.54
RARB	Transcription, proliferation and apoptosis	4.48	-	-5.42	-	-	-
RP 11-13N12.1	-	1.68	-2.12	-2.38	-1.82	-2.27	-1.87
RP11-467I20.6	-	2.42	-2.59	-2.89	-	-2.62	-2.23
RP11-807H7.1	-	1.54	-1.64	-	-	-	-
RP11-80B9.1	-	12.18	-21.29	-27.6	-	-8.98	-7.49
RP11-80B9.4	-	6.51	-7.15	-8.22	-	-6	-4.97
SNORA23	-	1.58	-	1.73	-	-	1.83
SNORD115-15	-	-1.9	-	-	-	-	2.58
SNORD115-42	-	-1.94	-	-2.13	-	-	2.65
SNORD115-6	-	-1.9	-	-2.36	-	-	2.7
TBX20	Angiogenesis, transcription and proliferation	1.53	-	-1.79	-	-	-
TFPI2	N/F	2.34	-	5.97	-	-	-
TIMP1	Proliferation	-1.73	-	1.52	-	2.35	1.74
TRDMT1	Epigenetic regulation of gene expression	1.64	-1.92	-1.62	-	-1.78	-
Total genes modulated		44	15	35	1	14	25

(-) There are no GOTERM_BP_FAT on the basis of data or no change in the fold change**, N/F**: No involved in cancer development according to the analysis.



Supplementary Fig. 1. Common altered genes between HPV 16 E6 variants. The Venn diagrams show comparison between variants pairs (a fold change ≥1.5 and ≤ -1.5). The p-value was calculated with FDR, p-value cut offs of <0.05 were used.

Supplementary Table S2. Overlapped genes of HPV16 E6 oncoprotein variants.

Gene	Cellular process	Fold change AA-a	Fold change AA-c	Fold change E-A176/G350	Fold change E-C188/G350	Fold change E-G350
ARID5B	Migration, signaling and transcription	-	1.65	-	-	1.56
CTNNA2	Adhesion and migration	-	-1.73	-	-	-1.57
EPHA5	Signaling	-	-4.37	-	-	-4.29
IGF1R	Apoptosis, migration, proliferation and signaling	1.59	-	-	-	1.75
KLF5	Angiogenesis, proliferation and transcription	-	1.69	-	-	-1.63
NID1	Adhesion	-	-2.28	-	-	7.6
PDGFC	Signaling	-	-2.18	-	5.43	-
ROR1	Signaling	2.5	-	-	-	1.98
SYNE1	Apoptosis	-	-4.87	-	-	-2.02
TGFBR2	Angiogenesis, differentiation, proliferation and signaling	-	2.93	-	2.3	-
THBS1	Adhesion, angiogenesis, apoptosis, cell cycle, migration, proliferation and signaling	-3.92	1.99	-	-	-
AC007740.1	N/F	3.81	13.5	-	-	2.39
AC016716.2	N/F	-	-1.92	-	-	-1.79
AC017002.2	N/F	-1.74	-	-	-	-1.79
BCHE	N/F	-	1.57	-	-	2.91
C11orf88	N/F	-1.74	2.13	-	-	-1.68
CRIM1	N/F	-	1.56	-	1.64	-
IGHD3-16	N/F	-	2.23	1.94	-	-
MAP2	N/F	-	1.52	-	2.16	4.98

MDM1	N/F	1.9	7.79	-	-	-
MIR181B1	N/F	-	-2.3	-	-2.06	-
RDH10	N/F	-1.91	-9.63	-	-	-
RNA5SP202	N/F	-	-	-	1.52	1.93
RNF150	N/F	-	1.85	-	-	1.84
RNU7-76P	N/F	-	-1.66	-	-	-1.54
RP11-11N9.4	N/F	-	2.73	-	1.88	-
RP11-284A20.1	N/F	-	-4.43	-	-4.02	-
RP11-642D21.1	N/F	-	-	-1.71	-1.58	-1.78
SNORA11	N/F	-	-	1.67	-	2.07
SNORA37	N/F	-	-	-	1.56	2.05
SNORA52	N/F	-	1.63	-	1.51	-
SNORD115-10	N/F	-	-	1.71	-	2.92
SNORD115-11	N/F	-	-	1.88	-	2.8
SNORD115-12	N/F	-	-1.97	1.77	-	2.93
SNORD115-21	N/F	-	-1.76	-	-	2.19
SNORD115-22	N/F	-	-	1.88	-	2.91
SNORD115-34	N/F	-	-2.15	-	-	3.39
SNORD115-40	N/F	-	-	1.62	-	2.32
SNORD115-43	N/F	-	-	1.88	-	2.8
SNORD115-44	N/F	-	-	1.56	-	2.35
SNORD115-5	N/F	-	-1.97	1.77	-	2.93
TENM1	N/F	4.93	2.36	-	-	-
TRIM2	N/F	-	-2.06	-	-1.72	-

(-) There are no GOTERM_BP_FAT on the basis of data or no change in the fold change**, N/F**: No involved in cancer development according to
the analysis.

Supplementary Table S3. Genes modulated by E6 HPV 16 oncoprotein expression of E6 variants vs. E-prototype in C-33A cells.

Biological process	HPV 16 variant	Upregulated genes (Fold change) vs. E-Prototype	Downregulated genes (Fold change) vs. E-Prototype
Adhesion	AA-a	CDH9 (1.9), NRCAM (4.11), COL11A1 (6.01)	**THBS1 (-3.92)**
	AA-c	PARD3-AS1 (1.55), ABL2 (1.59), CDH6 (1.69), BMPR1B (1.7), **THBS1 (1.99)**, CADM1 (2.68), SRPX (3.37)	SIRPA (-1.53), **CTNNA2 (-1.73)**, THY1 (-1.78), CDH18 (-2.01), PCDHB3 (-2.02), ITGA6 (-2.09), **NID1 (-2.28)**, PKP2 (-2.29)
	E-A176/ G350	-	-
	E-C188/ G350	-	-
	E-G350	EZR-AS1 (1.52), ROBO2 (1.59), PCDHB15 (1.61), CDH2 (1.65), CDK6 (1.81), PVRL3 (1.83), PCDH9 (2.45), **NID1 (7.6)**	**CTNNA2 (-1.57)**
Angiogenesis	AA-a	ANGPT1 (1.53)	**THBS1 (-3.92)**
	AA-c	**KLF5 (1.69), THBS1 (1.99), TGFBR2 (2.93)**	ANPEP (-1.51), FGF9 (-1.58), THY1 (-1.78), HDAC9 (-4.83)
	E-A176/ G350	-	-
	E-C188/ G350	**TGFBR2 (2.3)**	-
	E-G350	-	**KLF5 (-1.63)**
Apoptosis	AA-a	ANGPT1 (1.53), **IGF1R (1.59)**	**THBS1 (-3.92)**
	AA-c	NR4A1 (1.69), **THBS1 (1.99),** CADM1 (2.68), PEG10 (3.43)	SLC25A6 (-1.53), CD70 (-1.64),CUL5 (-1.68), CAMK1D (-1.96), HIPK2 (-2.66), EEF1A2 (-2.68), **SYNE1 (-4.87)**
	E-A176/ G350	-	-
	E-C188/ G350	-	-

	E-G350	BMP7 (1.54), BNIP3 (1.62), **IGF1R (1.75)**	**SYNE1 (-2.02)**
Cell cycle	AA-a	FAM5C (3.48)	**THBS1 (-3.92)**
	AA-c	PARD3-AS1(1.55), FBXO43 (1.58), CYP26B1 (1.64), **THBS1 (1.99)**	CUL5 (-1.68)
	E-A176/ G350	PROX1-AS1 (1.53)	-
	E-C188/ G350	-	-
	E-G350	BMP7 (1.54), CDK6 (1.81)	-
Differentiation	AA-a	NRCAM (4.11)	-
	AA-c	BMPR1B (1.7), **TGFBR2 (2.93)**	SMAP1 (-1.77), THY1 (-1.78), GLI2 (-1.99)
	E-A176/ G350	PROX1-AS1 (1.53)	-
	E-C188/ G350	**TGFBR2 (2.3)**	-
	E-G350	EZR-AS1 (1.52), BMP7 (1.54), CDK6 (1.81)	ROBO2 (1.59), EMP1 (-11.76)
Migration	AA-a	**IGF1R (1.59),** NRCAM (4.11)	**THBS1 (-3.92)**
	AA-c	**ARID5B (1.65), THBS1 (1.99),** HMGCR (3.17)	**CTNNA2 (-1.73),** THY1 (-1.78), ITGA6 (-2.09), HDAC9 (-4.83)
	E-A176/G350	-	-
	E-C188/G350	-	-
	E-G350	**ARID5B (1.56)**, CDH2 (1.65), **IGF1R (1.75)**	**CTNNA2 (-1.57)**
Proliferation	AA-a	**IGF1R (1.59)**	**THBS1 (-3.92)**
	AA-c	**KLF5 (1.69), THBS1 (1.99), TGFBR2 (2.93)**	FGF9 (-1.58), CD70 (-1.64), TIMP2 (-1.66), CUL5 (-1.68), BMI1 (-1.75), EMP3 (-1.77), GLI2 (-1.99), EMP2 (-2.08), PRKD1 (-2.36), HIPK2 (-2.66)
	E-A176/ G350	PROX1-AS1 (1.53)	-

	E-C188/ G350	ADAMTS1 (1.54), **TGFBR2 (2.3)**	-
	E-G350	BMP7 (1.54), **IGF1R (1.75)**, CDK6 (1.81), MAB21L2 (4.75)	**KLF5 (-1.63)**, EMP1 (-11.76)
Signaling	AA-a	ANGPT1 (1.53), **IGF1R (1.59), ROR1 (2.5)**	**THBS1 (-3.92)**
	AA-c	OR2G3 (1.53), ARL8B (1.55), PARD3-AS1(1.55), **ARID5B (1.65)**, CYP26B1 (1.64), BMPR1B (1.7), LRRN3 (1.8), FSHR (1.86), **THBS1 (1.99),** RAB3C (2.06), PLCH1 (2.11), **TGFBR2 (2.93),** HMGCR (3.17), PEG10 (3.43), CALCR (61.23)	ARRB1 (-1.51), FGF9 (-1.58), GABRE (-1.61), KIAA1324L (-1.79), GNAL (-1.84), **PDGFC (-2.18),** PRKD1 (-2.36), HIPK2 (-2.66), **EPHA5 (-4.37)**
	E-A176/ G350	-	-
	E-C188/ G350	OR12D3 (1.56), PRKACG (1.6), SLC12A2 (1.7), **TGFBR2 (2.3), PDGFC (5.43)**	-
	E-G350	BMP7 (1.54), **ARID5B (1.56)**, **IGF1R (1.75), ROR1 (1.98)**, SPRY2 (2.31)	**EPHA5 (-4.29)**
Translation	AA-a	-	-
	AA-c	DIO2 (2.09)	DNAJC1 (-1.78), EEF1A2 (-2.68)
	E-A176/ G350	-	-
	E-C188/ G350	-	-
	E-G350	-	-
Transcription	AA-a	SERTAD2 (2.07)	-
	AA-c	HABP4 (1.57), ZNF667-AS1 (1.58), ZNF542 (1.59), **ARID5B (1.65)**,	PARP14 (-1.51), NPAS3 (-1.53), ZNF607 (-1.6), NCOA3 (-1.63), ZNF600 (-1.67), BMI1 (-1.75),

	NR4A1 (1.69), **KLF5 (1.69),** RERE (1.85)	PDLIM1 (-1.77), ZNF253 (-1.8), GLI2 (-1.99), ZNF558 (-2.22), ZNF91 (-2.22), ZNF83 (-2.24), HIPK2 (-2.66), ZNF781 (-2.98), HDAC9 (-4.83)
E-A176/ G350	PROX1-AS1 (1.53)	-
E-C188/ G350	-	-
E-G350	BMP7 (1.54), **ARID5B (1.56)**, PLAG1 (1.56), CDCA7L (1.57), TRPS1 (1.57)	BACH1-IT2 (-1.54), EGR1 (-1.58), **KLF5 (-1.63)**

Genes marked with bold letters means altered expression by two HPV 16 variants. (-) No altered genes were found. Genes were grouped according to GOTERM -BP- FAT gene ontology databases. Overlapping genes between the E-Prototype and HPV 16 variants were not included.

Supplementary Table S4. Functional enrichment analysis of genes

GO term	AA-a vs. E-Prototype	AA-c vs. E-Prototype	E-A176/ G350 vs. E-Prototype	E-C188/ G350 vs. E-Prototype	E-G350 vs. E-Prototype
Actin binding	-	*KIAA1949, FMN2, FMNL2, LIMA1, SYNE1, MYH15, MYO1B*	-	-	-
Alternative splicing	-	-	-	*MUSK, PHF14, TRIM9, TRDMT1, NPFFR2*	-
Angiogenesis	-	*FGF9, TBX20, ANPEP, THY1*	-	-	-
Apoptosis	-	**PEG10, CADM1, THBS1, ITPR1**	-	-	-
Basement membrane	-	*FGF9, NID1, TIMP2*	-	-	-
Calmodulin-binding	-	-	-	-	**MAP2, SNTB1, PPP3CA**
Cell junction/ Cadherin/ WNT signaling	-	-	-	-	**EZR-AS1, PVRL3, PCDHB15, PCDH9, ROBO2, NID1, CDH2**
Cell membrane: topology	-	**CALCR, GCNT4, CADM1, HLA-DRB1, MAOA, TGFBR2, LRRN3, BET1, FSHR, ITPR1, CDH6, OR2G3, SLC1A3, SLC2A5, RNF150, SLC30A1, SLC16A9, KCNH7, HLA-DPA1, BMPR1B, HLA-**	-	-	-

		DOA, CRIM1			
Cell migration	-	**HMGCR, ARID5B, THBS1**	-	-	**ARID5B, ROBO2, CDH2, BMP7**
Cell projection	-	**CALCR, DNM3, SLC1A3, CADM1, MAP2**	-	-	**IGF1R, EZR-AS1, MAP2, BNIP3, CDK6, ROBO2, CDH2**
Cell projection organization	-	*DCC, LIMA1, SPAG6, ITGA6, GLI2, CTNNA2*	-	-	-
Cell-cell adhesion (NRCAM, CDH9, COL11A)	**NRCAM, CDH9, COL11A1**	-	-	-	-
Cell-cell adhesion (PKP2, CDH18, PCDHB3, PCDH7, THY1, CTNNA2)	-	*PKP2, CDH18, PCDHB3, PCDH7, THY1, CTNNA2*	-	-	-
Cell-cell signaling	-	*MUSK, FGF9, PCDHB3, CD70, GLI2, CTNNA2*	-	-	-
EGF-like domain	-	*FBLN1, CUBN, NID1*	-	-	-
Endocytosis	-	**DNM3, AP1S2, LRRN3, TGFBR2, LOC653653, THBS1**	-	-	-
Enzyme linked receptor protein signaling pathway	-	-	-	-	**IGF1R, ARID5B, ROR1, BMP7**
Glycoprotein - Transmembrane Receptor	-	*DCC, SLC38A2, FGF9, LPPR1, LRRC4B, IFI30, CD70, ANPEP, MUSK,*	-	*MUSK, CUBN, LPPR1, NPFFR2*	-

		NPFFR2, PDGFC, OLFM2, RECK, GABRE, KIAA1324L, CUBN, PCDHB3, NID1, PCDH7, SIRPA, THY1, ELFN1, EPHA5, FBLN1, SEMA6B, ITGA6, CDH18, CACNA1H, EMP3, EMP2			
Immune response	-	**CADM1, HLA-DRB1, HLA-DPA1, HLA-DOA**	-	-	-
Oxidation reduction - regulation of hormone levels	-	**ALDH1A1, SLC1A3, DIO2, HMGCR, MAOA, CYP26B1, BCO2, ENOX1**	-	-	-
Phosphoprotein/Zinc-finger	-	-	-	*FMN2, TRIM2, MUSK, PHF14, TRIM9, LPPR1*	-
Positive regulation - activation of kinase activity	-	-	-	**PRKACG, TGFBR2, PDGFC**	-
Positive regulation of apoptosis	-	*DCC, CUL5, HIPK2, CD70, RARB*	-	-	-
Positive regulation of cell división	-	*FGF9, PDGFC, MDK*	-	-	-
Protein kinase activity	**IGF1R, ROR1, MARK1**	-	-	-	-
Regulation of cell development	-	*MUSK, HDAC9, GLI2, TIMP2, THY1*	-	-	-
Regulation of cell proliferation	-	-	-	**TGFBR2, PDGFC, ADAMTS1, TIMP1**	-
Response to	-	**CALCR, BCHE, TGFBR2,**	-	-	-

endogenous stimulus		**HMGCS1, PPP3CA, THBS1**			
Steroid binding	-	-	-	-	**IGF1R, BCHE, PPP3CA, BMP7**
TGF-beta signaling pathway	-	**TGFBR2, BMPR1B, THBS1, BMP5**	-	-	-
Tyrosine kinase signaling pathway	**IGF1R, ROR1, ANGPT1**	-	-	-	-
Vesicular fraction	-	**HMGCR, MAP2, CYP26B1, PPP3CA, ITPR1**	-	-	**IGF1R, MAP2, PPP3CA**

Upregulated genes (bold), downregulated genes (italic (-) No altered genes were found. GO term: Gene Ontology term

Supplementary Table S5. Genes description modulated by E6 HPV16 variants expression

Gene	Gene function	Studies in cancer
ABL2	Non-receptor tyrosine-protein kinase that plays an ABL1-overlapping role in key processes linked to cell growth and survival such as cytoskeleton remodeling in response to extracellular stimuli, cell motility and adhesion and receptor endocytosis. Coordinates actin remodeling through tyrosine phosphorylation of proteins controlling cytoskeleton dynamics like MYH10 (involved in movement); CTTN (involved in signaling); or TUBA1 and TUBB (microtubule subunits). Binds directly F-actin and regulates actin cytoskeletal structure through its F-actin-bundling activity. Involved in the regulation of cell adhesion and motility through phosphorylation of key regulators of these processes such as CRK, CRKL, DOK1 or ARHGAP35. Adhesion-dependent phosphorylation of ARHGAP35 promotes its association with RASA1, resulting in recruitment of ARHGAP35 to the cell periphery where it inhibits RHO. Phosphorylates multiple receptor tyrosine kinases like PDGFRB and other substrates which are involved in endocytosis regulation such as RIN1. In brain, may regulate neurotransmission by phosphorylating proteins at the synapse. ABL2 acts also as a regulator of multiple pathological signaling cascades during infection. Pathogens can highjack ABL2 kinase signaling to reorganize the host actin cytoskeleton for multiple purposes, like facilitating intracellular movement and host cell exit. Finally, functions as its own regulator through autocatalytic activity as well as through phosphorylation of its inhibitor, ABI1.	Tissue of patients with hepatocellular carcinomas and adjacent tissues (Xing *et al.*, 2014).
ADAMTS1	Cleaves aggrecan, a cartilage proteoglycan, and may be involved in its turnover (By similarity). Has angiogenic inhibitor activity. Active metalloprotease, which may be associated with various inflammatory processes as well as development of cancer cachexia. May play a critical role in follicular ruptura.	Tissue of patients head and neck squamous cell carcinoma (Stokes *et al.*, 2010)
ANGPT1	Binds and activates TEK/TIE2 receptor by inducing its dimerization and tyrosine phosphorylation. Plays an important role in the regulation of angiogenesis, endothelial cell survival, proliferation, migration, adhesion and cell spreading,	Tissue of patients with oral squamous cell carcinoma (Jung *et al.*, 2015). Tissue of canine cortisol-secreting

	reorganization of the actin cytoskeleton, but also maintenance of vascular quiescence. Required for normal angiogenesis and heart development during embryogenesis. After birth, activates or inhibits angiogenesis, depending on the context. Inhibits angiogenesis and promotes vascular stability in quiescent vessels, where endothelial cells have tight contacts. In quiescent vessels, ANGPT1 oligomers recruit TEK to cell-cell contacts, forming complexes with TEK molecules from adjoining cells, and this leads to preferential activation of phosphatidylinositol 3-kinase and the AKT1 signaling cascades. In migrating endothelial cells that lack cell-cell adhesions, ANGT1 recruits TEK to contacts with the extracellular matrix, leading to the formation of focal adhesion complexes, activation of PTK2/FAK and of the downstream kinases MAPK1/ERK2 and MAPK3/ERK1, and ultimately to the stimulation of sprouting angiogenesis. Mediates blood vessel maturation/stability. Implicated in endothelial developmental processes later and distinct from that of VEGF. Appears to play a crucial role in mediating reciprocal interactions between the endothelium and surrounding matrix and mesenchyme.	adrenocortical tumor and normal adrenal glands (Kool *et al.,* 2014).
ANPEP	Broad specificity aminopeptidase. Plays a role in the final digestion of peptides generated from hydrolysis of proteins by gastric and pancreatic proteases. May play a critical role in the pathogenesis of cholesterol gallstone disease. May be involved in the metabolism of regulatory peptides of diverse cell types, responsible for the processing of peptide hormones, such as angiotensin III and IV, neuropeptides, and chemokines. Found to cleave antigen peptides bound to major histocompatibility complex class II molecules of presenting cells and to degrade neurotransmitters at synaptic junctions. Is also implicated as a regulator of IL-8 bioavailability in the endometrium, and therefore may contribute to the regulation of angiogenesis. Is used as a marker for acute myeloid leukemia and plays a role in tumor invasion. In case of human coronavirus 229E (HCoV-229E) infection, serves as receptor for HCoV-229E spike glycoprotein. Mediates as well human cytomegalovirus (HCMV) infection	Tissue samples: tumour from patients with metastatic prostate cancer (MPC), castrate-refractory prostate cancer (CRPC) samples, lymph node metastases (LNMs), cancer-adjacent nonmalignant (AN) prostate tissues, and benign prostatic hyperplasia (BPH) samples. Prostate cell line: LNCaP, DU145 and BPHI (Sørensen *et al.,* 2013). Normal colorectal epitelial and invasive tumor cells (Wiese *et al.,* 2007).

ARID5B	Transcription coactivator that binds to the 5'-AATA[CT]-3' core sequence and plays a key role in adipogenesis and liver development. Acts by forming a complex with phosphorylated PHF2, which mediates demethylation at Lys-336, leading to target the PHF2-ARID5B complex to target promoters, where PHF2 mediates demethylation of dimethylated 'Lys-9' of histone H3 (H3K9me2), followed by transcription activation of target genes. The PHF2-ARID5B complex acts as a coactivator of HNF4A in liver. Required for adipogenesis: regulates triglyceride metabolism in adipocytes by regulating expression of adipogenic genes.	-
ARL8B	May play a role in lysosome motility. May play a role in chromosome segregation.	-
ARRB1	Functions in regulating agonist-mediated G-protein coupled receptor (GPCR) signaling by mediating both receptor desensitization and resensitization processes. Acts as signaling scaffold for MAPK pathways such as MAPK1/3 (ERK1/2). Acts as signaling scaffold for the AKT1 pathway. Is involved in IGF1-stimulated AKT1 signaling leading to increased protection from apoptosis. Appears to function as signaling scaffold involved in regulation of MIP-1-beta-stimulated CCR5-dependent chemotaxis. Involved in attenuation of NF-kappa-B-dependent transcription in response to GPCR or cytokine stimulation by interacting with and stabilizing CHUK. Involved in regulation of LEF1 transcriptional activity via interaction with DVL1 and/or DVL2.	The human breast cancer cell lines: MDA-MB-468 and MDA-MB-231. Patients with primary invasive breast cancer and premenopausal breast cancer patients with invasive stage II disease (Lundgren *et al.*, 2011)
BACH1-IT2	-	-
BMI1	Component of a Polycomb group (PcG) multiprotein PRC1-like complex, a complex class required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. PcG PRC1 complex acts via chromatin remodeling and modification of histones; it mediates monoubiquitination of histone H2A 'Lys-119', rendering chromatin heritably changed in its expressibility. In the PRC1 complex, it is required to stimulate the E3 ubiquitin-protein ligase activity of RNF2/RING2	Cell lines AGS (gastric adenocarcinoma), NUGC4 (gastric lymph node), COLO201 (colon; derived from metastatic site), THP-1 (peripheral blood) and HCT116 (colorectal carcinoma) (Sugihara *et al.*, 2013).

BMP7	Induces cartilage and bone formation. May be the osteoinductive factor responsible for the phenomenon of epithelial osteogenesis. Plays a role in calcium regulation and bone homeostasis	Patients with urothelial carcinoma and healthy transitional epithelium (TE) of the urinary tract (Kuzaka *et al.*, 2015)
BMPR1B	On ligand binding, forms a receptor complex consisting of two type II and two type I transmembrane serine/threonine kinases. Type II receptors phosphorylate and activate type I receptors which autophosphorylate, then bind and activate SMAD transcriptional regulators.	Glioblastoma cell lines U251, U87, SF763. Glioblastoma cell line U-251 and normal human astrocytes. Athymic BALB/c nude mice (female) (Liu *et al.*, 2012). Tissue of patients with primary breast cancer tissue and non-affected normal breast tissue. Breast cancer cell line: MDA-MB-231 (Bokobza *et al.*, 2009) Tissue of patients with normal prostate and prostate tumor (Penney *et al.*, 2015).
BNIP3	Apoptosis-inducing protein that can overcome BCL2 suppression. May play a role in repartitioning calcium between the two major intracellular calcium stores in association with BCL2. Involved in mitochondrial quality control via its interaction with SPATA18/MIEAP: in response to mitochondrial damage, participates to mitochondrial protein catabolic process (also named MALM) leading to the degradation of damaged proteins inside mitochondria. The physical interaction of SPATA18/MIEAP, BNIP3 and BNIP3L/NIX at the mitochondrial outer membrane regulates the opening of a pore in the mitochondrial double membrane in order to mediate the translocation of lysosomal proteins from the cytoplasm to the mitochondrial matrix. Plays an important role in the calprotectin (S100A8/A9)-induced cell death pathway	-
CADM1	Mediates homophilic cell-cell adhesion in a Ca (2+)-independent manner. Also mediates heterophilic cell-cell adhesion with CADM3 and PVRL3 in a Ca (2+)-independent manner. Acts as a tumor suppressor in non-small-cell lung cancer (NSCLC) cells. Interaction with CRTAM promotes natural killer (NK) cell cytotoxicity and interferon-gamma (IFN-	NZB and C58 mice. Cell line: 6DT1 and Mvt-1 (Faraji *et al.*, 2012). Tissue of patients with cervical cancer, CIN1 and CIN3 lesions (Overmeer *et*

	gamma) secretion by CD8+ cells in vitro as well as NK cell-mediated rejection of tumors expressing CADM3 in vivo. May contribute to the less invasive phenotypes of lepidic growth tumor cells. CADM1, together with MITF, is essential for development and survival of mast cells in vivo. Acts as a synaptic cell adhesion molecule and plays a role in the formation of dendritic spines and in synapse assembly (By similarity). May be involved in neuronal migration, axon growth, pathfinding, and fasciculation on the axons of differentiating neurons.	*al.*, 2008)
CALCR	This is a receptor for calcitonin. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase. The calcitonin receptor is thought to couple to the heterotrimeric guanosine triphosphate-binding protein that is sensitive to cholera toxin	Biopsies of patients with normal pancreas, pancreatitis, pancreatic adenocarcinoma, pancreatic adenocarcinoma metastases, and pancreatic neuroendocrine tumors (Bloomston *et al.*, 2004)
CAMK1D	Calcium/calmodulin-dependent protein kinase that operates in the calcium-triggered CaMKK-CaMK1 signaling cascade and, upon calcium influx, activates CREB-dependent gene transcription, regulates calcium-mediated granulocyte function and respiratory burst and promotes basal dendritic growth of hippocampal neurons. In neutrophil cells, required for cytokine-induced proliferative responses and activation of the respiratory burst. Activates the transcription factor CREB1 in hippocampal neuron nuclei. May play a role in apoptosis of erythroleukemia cells. In vitro, phosphorylates transcription factor CREM isoform Beta	Tissue of patients with primary breast tumors, ductal carcinoma in situ and normal. Cell line: MCF10A (Bergamaschi *et al.*, 2008)
CD70	Cytokine that binds to CD27. Plays a role in T-cell activation. Induces the proliferation of costimulated T-cells and enhances the generation of cytolytic T-cells	Tissue of patients with renal cell carinoma and adjacent normal renal tissue. Cell lines: A498, ACHN, Caki-1, Caki-2, 786-O and HEK293T (Jilaveanu *et al.*, 2012).

CDCA7L	Plays a role in transcriptional regulation as a repressor that inhibits monoamine oxidase A (MAOA) activity and gene expression by binding to the promoter. Plays an important oncogenic role in mediating the full transforming effect of MYC in medulloblastoma cells. Involved in apoptotic signaling pathways; May act downstream of P38-kinase and BCL-2, but upstream of CASP3/caspase-3 as well as CCND1/cyclin D1 and E2F1	-
CDH18	Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types.	-
CDH2	Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types.	-
CDH6	Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types	Peripheral blood samples of patientes with renal cell carcinoma and normal (Shimazui *et al.*, 2004)
CDH9	Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types.	Cell lines: TK173, TK188, TK163, A-498, Caki-2, CRL-1611, CRL-1932, CRL-1933, MZ-1257, MZ-1851, MZ-1774, TW-33, BN-30, NH-99. Tissue of lung, liver, kidney, esophagus, colon, cérvix, brain, bladder, adipose, ovary, placenta, prostate, skeletal muscle, small intestine, spleen, testes, thymus, thyroid, trachea. (Thedieck *et al.*, 2007)

CDK6	Serine/threonine-protein kinase involved in the control of the cell cycle and differentiation; promotes G1/S transition. Phosphorylates pRB/RB1 and NPM1. Interacts with D-type G1 cyclins during interphase at G1 to form a pRB/RB1 kinase and controls the entrance into the cell cycle. Involved in initiation and maintenance of cell cycle exit during cell differentiation; prevents cell proliferation and regulates negatively cell differentiation, but is required for the proliferation of specific cell types (e.g. erythroid and hematopoietic cells).	Tissue of patients with carvical cáncer, high-grade squamous intraepithelial lesion, low-grade squamous intraepithelial lesions and normal cervix (Arvanitis *et al.*, 2008).
COL11A1	May play an important role in fibrillogenesis by controlling lateral growth of collagen II fibrils	Tissue of patients with pancreatic ductal adenocarcinomas, chronic pancreatitis and normal human pacreas (García-Pravia *et al.*, 2013).
CTNNA2	May function as a linker between cadherin adhesion receptors and the cytoskeleton to regulate cell-cell adhesion and differentiation in the nervous system. Regulates morphological plasticity of synapses and cerebellar and hippocampal lamination during development. Functions in the control of startle modulation (By similarity)	-
CUL5	Core component of multiple SCF-like ECS (Elongin-Cullin 2/5-SOCS-box protein) E3 ubiquitin-protein ligase complexes, which mediate the ubiquitination and subsequent proteasomal degradation of target proteins. As a scaffold protein may contribute to catalysis through positioning of the substrate and the ubiquitin-conjugating enzyme. The functional specificity of the E3 ubiquitin-protein ligase complex depends on the variable substrate recognition component. ECS (SOCS1) seems to direct ubiquitination of JAK2. Seems to be involved in proteosomal degradation of p53/TP53 stimulated by adenovirus E1B-55 kDa protein. May form a cell surface vasopressin receptor	Cell line: HMEC, MCF-10A, MCF-7 and MDA-MB-231.Normal and tumor tissue of breast, uterus, colon, stomach, ovary, cérvix, lung, kidney, rectum, small intestine, thyroid, prostate and pancreas (Fay *et al.*, 2003)

CYP26B1	Involved in the metabolism of retinoic acid (RA), rendering this classical morphogen inactive through oxidation. Involved in the specific inactivation of all-trans-retinoic acid (all-trans-RA), with a preference for the following substrates: all-trans-RA > 9-cis-RA > 13-cis-RA. Generates several hydroxylated forms of RA, including 4-OH-RA, 4-oxo-RA, and 18-OH-RA. Esential for postnatal survival. Plays a central role in germ cell development: acts by degrading RA in the developing testis, preventing STRA8 expression, thereby leading to delay of meiosis. Required for the maintenance of the undifferentiated state of male germ cells during embryonic development in Sertoli cells, inducing arrest in G0 phase of the cell cycle and preventing meiotic entry. Plays a role in skeletal development, both at the level of patterning and in the ossification of bone and the establishment of some synovial joints.	-
DIO2	Responsible for the deiodination of T4 (3, 5,3',5'-tetraiodothyronine) into T3 (3, 5,3'-triiodothyronine). Essential for providing the brain with appropriate levels of T3 during the critical period of development	Cell lines thyroid carcinoma and normal thyroid tissue (Arnaldi *et al.,* 2005).
DNAJC1	May modulate protein synthesis (By similarity)	Tissue of patients with primary cutaneous melanoma (Papalas *et al.,* 2010)
EEF1A2	This protein promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis	Tissue of patients with tumours ovarian and normal ovarían. Cell line: HL60, PE04, PE01, OAW28, 41M, OVCAR3, OVCAR4, A2780, SKOV3, 59M, OAW42, PEO16, PEO14, PEA1 and OVCAR5 (Tomlinson *et al.,* 2007). Cell line: DU-145, PC-3, LNCaP, LoVo DX, HepG2 and 22Rv1. Tissue of patients with prostate cáncer and normal prostate (Scaggiante *et al.,* 2012) Patients with gastric cancer and normal controls (Yang *et al.,* 2015)

EGR1	Transcriptional regulator. Recognizes and binds to the DNA sequence 5'-CGCCCCCGC-3'(EGR-site). Activates the transcription of target genes whose products are required for mitogenesis and differentiation	Patients with gastric cáncer and normal gastric (Zheng *et al.*, 2010).
EMP1	-	Tissue of pacients with colorectal cancer and adjacent normal. Cell lines: SW-480 (Sun *et al.*, 2014a). Tissue patients with oral squamous cell carcinoma and adjacent normal (Zhang *et al.*, 2011). Tissue of patients with breast carcinoma and normal tissues (Sun *et al.*, 2014b).
EMP2	.-	-
EMP3	Probably involved in cell proliferation and cell-cell interactions	Four invasive and four noninvasive mammary carcinoma cell lines (Evtimova *et al.*, 2013). Tissue of patients with neuroblastomas, gliomas and normal brain (Li *et al.*, 2007). Glioblastomas and non-neoplastic brain tissue (Scrideli *et al.*, 2008).
EPHA5	Receptor tyrosine kinase which binds promiscuously GPI-anchored ephrin-A family ligands residing on adjacent cells, leading to contact-dependent bidirectional signaling into neighboring cells. The signaling pathway downstream of the receptor is referred to as forward signaling while the signaling pathway downstream of the ephrin ligand is referred to as reverse signaling. Among GPI-anchored ephrin-A ligands, EFNA5 most probably constitutes the cognate/functional ligand for EPHA5	Tissue of patients with high risk, ovarían cervical and normal epithelium cells (Pejovic *et al.*, 2009). Tissue of patients with hepatocellular carcinoma and normal liver (Sun *et al.*, 2008).
EZR-AS1	-	.

FAM5C	Inhibits neuronal cell proliferation by negative regulation of the cell cycle transition. Promotes pituitary gonadotrope cell proliferation, migration and invasion, when overexpressed. May play a role in cell pituitary tumor development	Tissue of patients tongue squamous cell carcinoma and normal tongue (Kuroiwa *et al.,* 2009).
FBXO43	Required to establish and maintain the arrest of oocytes at the second meiotic metaphase until fertilization. Probably acts by inhibiting the anaphase-promoting complex/cyclosome (APC/C) ubiquitin ligase. Probably recognizes and binds to some phosphorylated proteins and promotes their ubiquitination and degradation (Probable)	-
FGF9	Plays an important role in the regulation of embryonic development, cell proliferation, cell differentiation and cell migration. May have a role in glial cell growth and differentiation during development, gliosis during repair and regeneration of brain tissue after damage, differentiation and survival of neuronal cells, and growth stimulation of glial tumors	Tissue of patients with multiple endocrine neoplasia type 1 and normal páncreas (Dilley *et al.,* 2005). Patients with non-small cell lung cáncer and non-cancerous lung tissues (Ohgino *et al.,* 2014).
FSHR	Receptor for follicle-stimulating hormone. The activity of this receptor is mediated by G proteins which activate adenylate cyclase	Tissue of patients breast cáncer and normal breast (Pleaneix *et al.,* 2015). Tissue of patients with sarcomas and non-neoplastic (Renner *et al.,* 2013).
GABRE	GABA, the major inhibitory neurotransmitter in the vertebrate brain, mediates neuronal inhibition by binding to the GABA/benzodiazepine receptor and opening an integral chloride channel	Cell lines: H1299, A549, H520, H460 and BEAS-2B. Tissue of patients non-smallcell lung cáncer and non-cancerous. Tissue of brain (Zhang *et al.,* 2013).
GLI2	Acts as a transcriptional activator. May play a role during embryogenesis. Binds to the DNA sequence 5'-GAA-cCACCCA-3' which is part of the TRE-2S regulatory element that augments the Tax-dependent enhancer of human T-cell leukemia virus type 1. Is involved in the smoothened (SHH) signaling pathway.	-

GNAL	Guanine nucleotide-binding proteins (G proteins) are involved as modulators or transducers in various transmembrane signaling systems. G (olf) alpha mediates signal transduction within the olfactory neuroepithelium and the basal ganglia. May be involved in some aspect of visual transduction, and in mediating the effect of one or more hormones/neurotransmitters	-
HABP4	May be involved in nuclear functions such as the remodeling of chromatin and the regulation of transcription	-
HDAC9	Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4). Histone deacetylation gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression and developmental events. Represses MEF2-dependent transcription Function: Isoform 3 lacks active site residues and therefore is catalytically inactive. Represses MEF2-dependent transcription by recruiting HDAC1 and/or HDAC3. Seems to inhibit skeletal myogenesis and to be involved in heart development. Protects neurons from apoptosis, both by inhibiting JUN phosphorylation by MAPK10 and by repressing JUN transcription via HDAC1 recruitment to JUN promoter	Tissue of patients with adenocarcinoma, squamous cell carcinoma, large cell carcinoma. Cell lines: G12, V12, H61, A549, H358, H2087, H1819, H441, H1299, H820, HEK293T, LU130, LU135, LU139, LU140, TKB4, TKB5, TKB6, TKB1, TKB2, TKB7, TKB8, TKB14, TKB15, TKB16, TKB17 and TKB20 (Okudela *et al.*, 2014)
HIPK2	Serine/threonine-protein kinase involved in transcription regulation, p53/TP53-mediated celular apoptosis and regulation of the cell cycle. Acts as a corepressor of several transcription factors, including SMAD1 and POU4F1/Brn3a and probably NK homeodomain transcription factors. Phosphorylates PDX1, ATF1, PML, p53/TP53, CREB1, CTBP1, CBX4, RUNX1, EP300, CTNNB1, HMGA1 and ZBTB4. Inhibits cell growth and promotes apoptosis through the activation of p53/TP53 both at the transcription level and at the protein level (by phosphorylation and indirect acetylation). The phosphorylation of p53/TP53 may be mediated by a p53/TP53-HIPK2-AXIN1 complex. Involved in the response to hypoxia by acting as a transcriptional co-suppressor of HIF1A. Mediates transcriptional activation of TP73. In response to TGFB, cooperates with DAXX to activate JNK. Negative regulator through phosphorylation and subsequent proteasomal degradation of CTNNB1 and the antiapoptotic factor CTBP1. In the Wnt/beta-catenin	Tissue of patients with actinic keratosis, Bowen´s disease, keratoacanthoma, squamous cell carcinoma and basal cell carcinoma (Kwon *et al.*, 2015).

	signaling pathway acts as an intermediate kinase between MAP3K7/TAK1 and NLK to promote the proteasomal degradation of MYB. Phosphorylates CBX4 upon DNA damage and promotes its E3 SUMO-protein ligase activity. Activates CREB1 and ATF1 transcription factors by phosphorylation in response to genotoxic stress. In response to DNA damage, stabilizes PML by phosphorylation. PML**,** HIPK2 and FBXO3 may act synergically to actívate p53/TP53-dependent transactivation. Phosphorylation of RUNX1 and EP300 stimulates EP300 transcription regulation activity. Triggers ZBTB4 protein degradation in response to DNA damage. Modulates HMGA1 DNA-binding affinity. In response to high glucose, triggers phosphorylation-mediated subnuclear localization shifting of PDX1.	
HMGCR	Transmembrane glycoprotein that is the rate-limiting enzyme in cholesterol biosynthesis as well as in the biosynthesis of nonsterol isoprenoids that are essential for normal cell function including ubiquinone and geranylgeranyl proteins	-
IGF1R	Receptor tyrosine kinase which mediates actions of insulin-like growth factor 1 (IGF1). Binds IGF1 with high affinity and IGF2 and insulin (INS) with a lower affinity. The activated IGF1R is involved in cell growth and survival control. IGF1R is crucial for tumor transformation and survival of malignant cell. Ligand binding activates the receptor kinase, leading to receptor autophosphorylation, and tyrosines phosphorylation of multiple substrates, that function as signaling adapter proteins including, the insulin-receptor substrates (IRS1/2), Shc and 14-3-3 proteins. Phosphorylation of IRSs proteins lead to the activation of two main signaling pathways: the PI3K-AKT/PKB pathway and the Ras-MAPK pathway. The result of activating the MAPK pathway is increased celular proliferation, whereas activating the PI3K pathway inhibits apoptosis and stimulates protein synthesis. Phosphorylated IRS1 can activate the 85 kDa regulatory subunit of PI3K (PIK3R1), leading to activation of several downstream substrates, including protein AKT/PKB. AKT phosphorylation, in turn, enhances protein synthesis through mTOR activation and triggers the antiapoptotic effects of IGFIR through phosphorylation and inactivation of BAD. In parallel to PI3K-driven signaling, recruitment of Grb2/SOS by phosphorylated IRS1 or Shc leads to recruitment of Ras and activation of the ras-MAPK pathway. In addition to these two main signaling pathways IGF1R signals also through the Janus kinase/signal transducer and activator of transcription pathway (JAK/STAT).Phosphorylation of JAK proteins can lead to phosphorylation/activation of	Tissue of patients with stage II breast carcinoma and postmenopausal (Aaltonen *et al.,* 2014)

	signal transducers and activators of transcription (STAT) proteins. In particular activation of STAT3, may be essential for the transforming activity of IGF1R**.** The JAK/STAT pathway activates gene transcription and may be responsible for the transforming	
ITGA6	Integrin alpha-6/beta-1 is a receptor for laminin on platelets. Integrin alpha-6/beta-4 is a receptor for laminin in epithelial cells and it plays a critical structural role in the hemidesmosome	Stage I, III and VI breast cancer patients (Klahan *et al.*, 2014)
KIAA1324L	-	-
KLF5	Transcription factor that binds to GC box promoter elements. Activates the transcription of these genes.	Tissue of patients histologically normal epithelium and breast cancer (Zubor *et al.*, 2015). Cell lines: bladder (TSU-Prl) and breast cancer (HCC1937) (Ge *et al.*, 2014). Ovarian cancer cell line SKOV3 (Dong *et al.*, 2013).
LRRN3	-	Patients with pheochromocytomas (benign and sporadic, benign and hereditary, malignant) (Suh *et al.*, 2009).
MAB21L2	Required for several aspects of embryonic development including normal development of the eye (By similarity)	Tissue patientes with early intestinal tumours (human and mouse adenomas) and normal bowel (Segditsas *et al*, 2008).
NCOA3	Nuclear receptor coactivator that directly binds nuclear receptors and stimulates the transcriptional activities in a hormone-dependent fashion. Plays a central role in creating a multisubunit coactivator complex, which probably acts via remodeling of chromatin. Involved in the coactivation of different nuclear receptors, such as for steroids (GR and ER), retinoids (RARs and RXRs), thyroid hormone (TRs), vitamin D3 (VDR) and prostanoids (PPARs). Displays histone acetyltransferase activity. Also involved in the	Tissue of patients with breast cáncer (Burandt *et al.*, 2013). Tissue of patients with colorectal cáncer and adjacent normal (Li *et al.*, 2012).

	coactivation of the NF-kappa-B pathway via its interaction with the NFKB1 subunit. Interacts with PSMB9	
NID1	Sulfated glycoprotein widely distributed in basement membranes and tightly associated with laminin. Also binds to collagen IV and perlecan. It probably has a role in cell-extracellular matrix interactions	Tissue of patient with benign nevi, primarymelanoma and normal skin. HapMap CEU cell lines (Nan *et al.*, 2011). Plasma and tissue of patientes with ovarian serous cáncer (ovarian carcinoma at early (1/2) and late (3/4) stage) and healthy individuals (Li *et al.*, 2015)
NPAS3	May play a broad role in neurogenesis. May control regulatory pathways relevant to schizophrenia and to psychotic illness (By similarity)	-
NR4A1	Orphan nuclear receptor. May act concomitantly with NURR1 in regulating the expression of delayed-early genes during liver regeneration. Binds the NGFI-B response element (NBRE) 5'-AA-AAGGTCA-3' (By similarity). May inhibit NF-kappa-B transactivation of IL2. Participates in energy homeostasis by sequestrating the kinase STK11 in the nucleus, thereby attenuating cytoplasmic AMPK activation	Cell lines: MA-10, TM3, PLC, ILC, MLC, 15P-1, MSC-1, TM4, Testis. CD-1 mice (Robert *et al.*, 2006).
NRCAM	Cell adhesion, ankyrin-binding protein involved in neuron-neuron adhesion. May play a role in the molecular assembly of the nodes of Ranvier (By similarity)	Tissue of patients with ovarian carcinoma/primary peritoneal carcinoma and breast carcinoma (Davidson *et al.*, 2011)
OR12D3	Odorant receptor (Potential)	-
OR2G3	Odorant receptor (Potential)	-
PARD3-AS1	-	-
PARP14	Enhances STAT6-dependent transcription (By similarity). Has ADP-ribosyltransferase activity	-
PCDH9	Potential calcium-dependent cell-adhesion protein	Tissue of patients with gastric cáncer, primary tumors, nodal, hepatic

		metastatic and normal (Chen *et al.*, 2015) Primary gliomas and normal brain (Wang *et al.*, 2012)
PCDHB15	Potential calcium-dependent cell-adhesion protein. May be involved in the establishment and maintenance of specific neuronal connections in the brain	-
PCDHB3	Potential calcium-dependent cell-adhesion protein. May be involved in the establishment and maintenance of specific neuronal connections in the brain	-
PDGFC	Growth factor that plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis. Potent mitogen and chemoattractant for cells of mesenchymal origin. Required for normal skeleton formation during embryonic development, especially for normal development of the craniofacial skeleton and for normal development of the palate. Required for normal skin morphogenesis during embryonic development. Plays an important role in wound healing, where it appears to be involved in three stages: inflammation, proliferation and remodeling. Plays an important role in angiogenesis and blood vessel development. Involved in fibrotic processes, in which transformation of interstitial fibroblasts into myofibroblasts plus collagen deposition occurs. The CUB domain has mitogenic activity in coronary artery smooth muscle cells, suggesting a role beyond the maintenance of the latency of the PDGF domain. In the nucleus, PDGFC seems to have additional function.	Tissue of prostate with tumor and matastatic tumor (Chandran *et al.*, 2007)
PDLIM1	Cytoskeletal protein that may act as an adapter that brings other proteins (like kinases) to the cytoskeleton	-
PEG10	Prevents apoptosis in hepatocellular carcinoma (HCC) cells through interaction with SIAH1, a mediator of apoptosis. May also have a role in cell growth promotion and hepatoma formation. Inhibits the TGF-beta signaling by interacting with the TGF-beta receptor ALK1. When overexpressed, induces the formation of cellular extension, such as filipodia in association with ALK1. Involved at the immediate early stage of adipocyte differentiation (By similarity). May bind to the 5-GCCTGTCTTT-3 DNA sequence of the MB1 domain in the myelin basic protein (MBP) promoter (By similarity).	Tissue of patients with hepatocellular carcinoma and normal liver (Bang *et al.*, 2015). Cell line HepG2 and normal liver (Dong *et al.*, 2009)

PKP2	May play a role in junctional plaques	Bladder cancer cell lines: 5637, J82 and HUCs (Takahashi *et al.,* 2012) Tissue patients with gastric cancer and tissue normal gástric (Demirag *et al.*, 2011).
PLAG1	Transcription factor whose activation results in up-regulation of target genes, such as IGFII, leading to uncontrolled cell proliferation: when overexpressed in cultured cells, higher proliferation rate and transformation are observed. Other target genes such as CRLF1, CRABP2, CRIP2, PIGF are strongly induced in cells with PLAG1 induction. Proto-oncogene whose ectopic expression can trigger the development of pleomorphic adenomas of the salivary gland and lipoblastomas. Overexpression is associated with up-regulation of IGFII, is frequently observed in hepatoblastoma, common primary liver tumor in childhood. Cooperates with CBFB-MYH11, a fusion gene important for myeloid leukemia	Patients with thyroid tumors (follicular adenomas, papillary carcinomas, folicular variants and folicular thyroid carcinomas), tissue samples uterine leiomyomas. Cell line of breast cáncer: MCF-7 (Klemke *et al.*, 2014). Placental tissue samples from pregnant women with fetal growth restriction and normal pregnancies (Tang *et al.,* 2013). Adjacent non-neoplastic liver tissue of hepatoblastoma patients, normal fetal liver tissue of different fetal ages and hepatoblastoma tumors. Hepatoblastoma cell lines: HepT1, HepT2, HepT3, HepT4, HepG2, HepT6 and HUH6 (Zatkova *et al*, 2004).
PLCH1	The production of the second messenger molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) is mediated by calcium-activated phosphatidylinositol-specific phospholipase C enzymes.	-
PRKACG	Phosphorylates a large number of substrates in the cytoplasm and the nucleus	-

PRKD1	Serine/threonine-protein kinase that converts transient diacylglycerol (DAG) signals into prolonged physiological effects downstream of PKC, and is involved in the regulation of MAPK8/JNK1 and Ras signaling, Golgi membrane integrity and trafficking, cell survival through NF-kappa-B activation, cell migration, cell differentiation by mediating HDAC7 nuclear export, cell proliferation via MAPK1/3 (ERK1/2) signaling, and plays a role in cardiac hypertrophy, VEGFA-induced angiogenesis, genotoxic-induced apoptosis and flagellin-stimulated inflammatory response. Phosphorylates the epidermal growth factor receptor (EGFR) on dual threonine residues, which leads to the suppression of epidermal growth factor (EGF)-induced MAPK8/JNK1 activation and subsequent JUN phosphorylation. Phosphorylates RIN1, inducing RIN1 binding to 14-3-3 proteins YWHAB, YWHAE and YWHAZ and increased competition with RAF1 for binding to GTP-bound form of Ras proteins (NRAS, HRAS and KRAS). Acts downstream of the heterotrimeric G-protein beta/gamma-subunit complex to maintain the structural integrity of the Golgi membranes, and is required for protein transport along the secretory pathway. In the trans-Golgi network (TGN), regulates the fission of transport vesicles that are on their way to the plasma membrane. May act by activating the lipid kinase phosphatidylinositol 4-kinase beta (PI4KB) at the TGN for the local synthesis of phosphorylated inositol lipids, which induces a sequential production of DAG, phosphatidic acid (PA) and lyso-PA (LPA) that are necessary for membrane fission and generation of specific transport carriers to the cell surface. Under oxidative stress, is phosphorylated at Tyr-463 via SRC-ABL1 and contributes to cell survival by activating IKK complex and subsequent nuclear translocation and activation of NFKB1. Involved in cell migration by regulating integrin alpha-5/beta-3 recycling and promoting its recruitment in newly forming focal adhesión. Plays an important role in the proliferative response induced by low calcium in keratinocytes, through sustained activation of MAPK1/3 (ERK1/2) pathway. In epitelial cells, is required for transducing flagellin-stimulated inflammatory responses by binding and phosphorylating TLR5, which contributes to MAPK14/p38 activation and production of inflammatory cytokines. May play a role in inflammatory response by mediating activation of NF-kappa-B. May be involved in pain transmission by directly modulating TRPV1 receptor	Tissue of patients with primary squamous cell laryngeal cancinoma (Fountzilas *et al.*, 2013). Tissue of patients with normal breast from reduction mammoplasty and paired samples of histologically normal and ductal carcinoma in situ. Breast cell line: MCF7 (Hannafon *et al.*, 2011).
PROX1-AS1	-	-

PVRL3	Plays a role in cell-cell adhesion through heterophilic trans-interactions with nectin-like proteins or nectins, such as trans-interaction with PVRL2/nectin-2 at Sertoli-spermatid junctions. Trans-interaction with PVR induces activation of CDC42 and RAC small G proteins through common signaling molecules such as SRC and RAP1. Also involved in the formation of cell-cell junctions, including adherens junctions and synapses. Induces endocytosis-mediated down-regulation of PVR from the cell surface, resulting in reduction of cell movement and proliferation. Plays a role in the morphology of the ciliary body	-
RAB3C	Protein transport. Probably involved in vesicular traffic (By similarity)	-
RERE	Plays a role as a transcriptional repressor during development. May play a role in the control of cell survival. Overexpression of RERE recruits BAX to the nucleus particularly to POD and triggers caspase-3 activation, leading to cell death	-
ROBO2	Receptor for SLIT2, and probably SLIT1, which are thought to act as molecular guidance cue in celular migration, including axonal navigation at the ventral midline of the neural tube and projection of axons to different regions during neuronal development	Tissue of patients with prostate cancer and tissue of prostate normal (Choi *et al.*, 2014).
ROR1	Tyrosine-protein kinase receptor whose role is not yet clear	Tissue of patients with ovarian cancer and normal ovarian (Huilin *et al.*, 2014) Cell lines 380, 697, KASUMI-2, KOPN-8, MHHCALL-3, NALM-6, RCH-ACV, RS4; 11, SEM, SUP-B15, MOLT-16, CA-46 and REH. Tissue patients normal and patients acute lymphoblastic leukemia (Dave *et al.*, 2012)
SERTAD2	Acts at E2F-responsive promoters as coregulator to integrate signals provided by PHD- and/or bromodomain-containing transcription factors. May act as coactivator as well as	-

	corepressor of E2F1-TFDP1 and E2F4-TFDP1 complexes on E2F consensus binding sites, which would activate or inhibit E2F-target genes expression. Modulates fat storage by down-regulating the expression of key genes involved in adipocyte lipolysis, thermogenesis and oxidative metabolism	
SIRPA	Immunoglobulin-like cell surface receptor for CD47. Acts as docking protein and induces translocation of PTPN6, PTPN11 and other binding partners from the cytosol to the plasma membrane. Supports adhesion of cerebellar neurons, neurite outgrowth and glial cell attachment. May play a key role in intracellular signaling during synaptogenesis and in synaptic function (By similarity). Involved in the negative regulation of receptor tyrosine kinase-coupled cellular responses induced by cell adhesion, growth factors or insulin. Mediates negative regulation of phagocytosis, mast cell activation and dendritic cell activation. CD47 binding prevents maturation of immature dendritic cells and inhibits cytokine production by mature dendritic cells	Sample bone marrow and peripheral blood of breast cancer patients and normal controls. Breast cancer lines: CRL1500, MCF7, MRK-nul, YMB1, YMB1E, SKBR3 and MDA-MB-231 (Nagahara *et al.,* 2010).
SLC12A2	Electrically silent transporter system. Mediates sodium and chloride reabsorption. Plays a vital role in the regulation of ionic balance and cell volume	-
SLC25A6	Catalyzes the exchange of cytoplasmic ADP with mitochondrial ATP across the mitochondrial inner membrane. May participate in the formation of the permeability transition pore complex (PTPC) responsible for the release of mitochondrial products that triggers apoptosis.	-
SMAP1	GTPase activating protein that acts on ARF6. Plays a role in clathrin-dependent endocytosis. May play a role in erythropoiesis (By similarity)	Surgical specimens of pleomorphic malignant fibrous histiocytomas and myxofibrosarcomas (Takahashi *et al.,* 2006).
SPRY2	May function as an antagonist of fibroblast growth factor (FGF) pathways and may negatively modulate respiratory organogenesis	-
SRPX	May be involved in phagocytosis during disk shedding, cell adhesion to cells other	Tissue of patients with colon cancer

	than the pigment epithelium or signal transduction	and adjacent normal tissues. (Liu *et al.*, 2015). Surgical specimens of cancer tissue and adjacent normal mucosa of patients with gastric cancer. Gastric cancer lines: MKN1, MKN7, MKN45, MKN74, NUGC-3 and NUGC-4 (Yamada *et al.*, 2015).
SYNE1	Multi-isomeric modular protein which forms a linking network between organelles and the actin cytoskeleton to maintain the subcellular spatial organization. Component of SUN-protein-containing multivariate complexes also called LINC complexes which link the nucleoskeleton and cytoskeleton by providing versatile outer nuclear membrane attachment sites for cytoskeletal filaments. May be involved in the maintenance of nuclear organization and structural integrity. Connects nuclei to the cytoskeleton by interacting with the nuclear envelope and with F-actin in the cytoplasm. May be required for centrosome migration to the apical cell surface during early ciliogenesis	Cell lines: Coav-3, NIH:OVCAR-3, SK-OV-3, MDAH-2774, GG, HeSt, MT, HeLa S3, Calu-3, Calu-6, MCF7, Caco-2, Colo 320DM. Various types of tissue carcinoma and normal tissue (Marme *et al.*, 2008)
TGFBR2	Transmembrane serine/threonine kinase forming with the TGF-beta type I serine/threonine kinase receptor, TGFBR1, the non-promiscuous receptor for the TGF-beta cytokines TGFB1, TGFB2 and TGFB3. Transduces the TGFB1, TGFB2 and TGFB3 signal from the cell surface to the cytoplasm and is thus regulating a plethora of physiological and pathological processes including cell cycle arrest in epithelial and ematopoietic cells, control of mesenchymal cell proliferation and differentiation, wound healing, extracellular matrix production, immunosuppression and carcinogenesis. The formation of the receptor complex composed of 2 TGFBR1 and 2 TGFBR2 molecules symmetrically bound to the cytokine dimer results in the phosphorylation and the activation of TGFRB1 by the constitutively active TGFBR2. Activated TGFBR1 phosphorylates SMAD2 which dissociates from the receptor and interacts with SMAD4. The SMAD2-SMAD4 complex is subsequently translocated to the nucleus where it modulates the transcription of the TGF-beta-regulated genes. This constitutes the canonical SMAD-dependent TGF-beta signaling cascade. Also involved in non-canonical, SMAD-independent TGF-beta signaling pathways	Tissue of patients with oral cancers and normal tissue. (Sivadas *et al.*, 2015)

THBS1	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. Binds heparin. May play a role in dentinogenesis and/or maintenance of dentin and dental pulp (By similarity). Ligand for CD36 mediating antiangiogenic properties. Plays a role in ER stress response, via its interaction with the activating transcription factor 6 alpha (ATF6) which produces adaptive ER stress response factors (By similarity)	Tissue of patients histologically normal epithelium and breast cancer (Zubor *et al.*, 2015).
THY1	May play a role in cell-cellor cell-ligand interactions during synaptogenesis and other events in the brain.	Tissue of patients histologically confirmed resected hepatocellular carcinoma and paired non-tumor tissue samples of human hepatocellular carcinoma (Lu *et al.*, 2011). The parental cell line: SKOV-3. Ovarian cancer cell line: NIH: OVCAR-3, Caov-3, OW-1, HOC8. Ovarian cancer cell lines: MLS/P and GR (Abeysinghe *et al.*, 2003)
TIMP2	Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them by binding to their catalytic zinc cofactor. Known to act on MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, MMP-14, MMP-15, MMP-16 and MMP-19.	-
TRPS1	Transcriptional repressor. Binds specifically to GATA sequences and represses expression of GATA-regulated genes at selected sites and stages in vertebrate development. Regulates chondrocyte proliferation and differentiation. Executes multiple functions in proliferating chondrocytes, expanding the region of distal chondrocytes, activating proliferation in columnar cells and supporting the differentiation of columnar into hypertrophic chondrocytes	Cell lines: PC-3, LNCaP, DU145, 22rv1, NCI-H660, SK-Br-3, ZR75-1, MCF-7, MDA436, EFM19 and T47D. Tissue of patients with prostate cáncer and benign prostate hyperplasias (Suvinainen *et al.*, 2004)
ZNF253	May function as a transcription factor. Seem to have a transcriptional repression activity	-
ZNF542	-	-

ZNF558	May be involved in transcriptional regulation	-
ZNF600	May be involved in transcriptional regulation	Tissue of patients with clear cell renal cell carcinoma and normal kidney (Eckel-Passow *et al.*, 2014)
ZNF607	May be involved in transcriptional regulation	-
ZNF667-AS1	-	-
ZNF781	May be involved in transcriptional regulation	-
ZNF83	May be involved in transcriptional regulation	Tissue of patientes with colorectal cáncer and normal tissues (Jovov *et al.*, 2012). Tissue of patients African American and European American with hepatocellular carcinoma and normal liver.Cell line: HepG2 (Dong *et al.*, 2009).
ZNF91	-	Patients with stage IV Ovarian Clear Cell Carcinoma (Abelson *et al.*, 2013). Samples from patients with primary and recurrent operable gastrointestinal stromal tumors (Rink *et al.*, 2009).

Function genes were obtained from UniProtKB/Swiss-Prot data. Information about studies in cancer was obtained from Pubmed using the gene name followed by the word cancer. (-) No gene information was found.

References

Aaltonen, K.E., Rosendahl, A.H., Olsson, H., Malmström, P., Hartman, L., Fernö, M., 2014. Association between insulin-like growth factor-1 receptor (IGF1R) negativity and por prognosis in a cohort of women with primary breast cancer. BMC Cancer. 14, 794. doi: 10.1186/1471-2407-14-794.

Abelson, S., Shamai, Y., Berger, L., Skorecki, K., Tzukerman, M., 2013. Niche-dependent gene expression profile of intratumoral heterogeneous ovarian cancer stem cell populations. PLoS One. 8 (12), e83651. doi: 10.1371/journal.pone.0083651.

Abeysinghe, H.R., Cao, Q., Xu, J., Pollock, S., Veyberman, Y., Guckert, N.L., Keng, P., Wang, N., 2003. THY1 expression is associated with tumor suppression of human ovarian cancer. Cancer Genet Cytogenet. 143 (2), 125-132. dio: 10.1016/S0165-4608(02)00855-5.

Arnaldi, L.A., Borra, R.C., Maciel, R.M., Cerutti, J.M., 2005. Gene expression profiles reveal that DCN, DIO1, and DIO2 are underexpressed in benign and malignant thyroid tumors. Thyroid.15 (3), 210-221. doi:10.1089/thy.2005.15.210.

Arvanitis, D.A, Spandidos, D.A., 2008. Deregulation of the G1/S phase transition in cancer and squamous intraepithelial lesions of the uterine cervix: a case control study. Oncol Rep. 20 (4), 751-760. dio: dx.doi.org/10.3892/or_00000070.

Bergamaschi, A., Kim, Y.H., Kwei, K.A., La Choi, Y., Bocanegra, M., Langerød, A., Han, W., Noh, D.Y., Huntsman, D.G., Jeffrey, S.S., Børresen-Dale, A.L., Pollack, J.R., 2008. CAMK1D amplification implicated in epithelial-mesenchymal transition in basal-like breast cancer. Mol Oncol. 2 (4), 327-339. doi: 10.1016/j.molonc.2008.09.004.

Bloomston, M., Durkin, A., Yang, I., Rojiani, M., Rosemurgy, A.S., Enkmann, S., Yeatman, T.J., Zervos, E.E., 2004. Identification of molecular markers specific for pancreatic neuroendocrine tumors by genetic profiling of core biopsies. Ann Surg Oncol. 11 (4), 413-419.

Bokobza, S.M., Ye, L., Kynaston, H.E., Mansel, R.E., Jiang, W.G., 2009. Reduced expression of BMPR-IB correlates with por prognosis and increased proliferation of breast cancercells. Cancer Genomics Proteomics. 6 (2), 101-108.

Burandt, E., Jens, G., Holst, F., Jänicke, F., Müller, V., Quaas, A., Choschzick, M., Wilczak, W., Terracciano, L., Simon, R., Sauter, G., Lebeau, A., 2013. Prognostic relevance of AIB1 (NCoA3) amplification and overexpression in breast cancer. Breast Cancer Res Treat. 137 (3), 745-753. doi: 10.1007/s10549-013-2406-4.

Chandran, U.R., Ma, C., Dhir, R., Bisceglia, M., Lyons-Weiler, M., Liang, W., Michalopoulos, G., Becich, M., Monzon, F.A., 2007. Gene expression profiles of prostate cáncer reveal involvement of multiple molecular pathways in the metastaticprocess. BMC Cancer. 7, 64. doi: 10.1186/1471-2407-7-64.

Chen, Y., Xiang, H., Zhang, Y., Wang, J., Yu, G., 2015. Loss of PCDH9 is associated with the differentiation of tumor cells and metastasis and predicts poor survival in gastric cancer. Clin Exp Metastasis. 32 (5), 417-428. doi: 10.1007/s10585-015-9712-7.

Choi, Y.J., Yoo, N.J., Lee, S.H., 2014. Down-regulation of ROBO2 expression in prostate cancers. Pathol Oncol Res. 20 (3):517-519. doi: 10.1007/s12253-013-9722-1.

Dave, H., Anver, M.R., Butcher, D.O., Brown, P., Khan, J., Wayne, A.S., Baskar, S., Rader, C., 2012. Restricted cell surface expression of receptor tyrosine kinase ROR1 in pediatric B-lineage acute lymphoblasticleukemia suggests targetabilit with

therapeutic monoclonal antibodies. PLoS One. 7 (12), e52655. doi: 10.1371/journal.pone.0052655.

Davidson, B., Stavnes, H.T., Holth, A., Chen, X., Yang, Y., Shih, IeM.,Wang, T.L., 2011. Gene expression signatures differentiate ovarian/peritoneal serous carcinoma from breast carcinoma ineffusions. J Cell Mol Med. 15 (3), 535-544. doi: 10.1111/j.1582-4934.2010.01023.x.

Demirag, G.G., Sullu, Y., Gurgenyatagi, D., Okumus, N.O., Yucel, I., 2011.Expression of plakophilins (PKP1, PKP2, and PKP3) in gastric cancers. Diagn Pathol. 6, 1. doi: 10.1186/1746-1596-6-1.

Dilley, W.G., Kalyanaraman, S., Verma, S., Cobb, J.P., Laramie, J.M., Lairmore, T.C., 2005. Global gene expression in neuroendocrine tumors from patients with the MEN1 syndrome. Mol Cancer. 4 (1), 9. doi: 10.1186/1476-4598-4-9.

Dong, H., Ge, X., Shen, Y., Chen, L., Kong, Y., Zhang, H., Man, X., Tang, L., Yuan, H., Wang, H., Zhao, G., Jin, W., 2009. Gene expression profile analysis of human hepatocellular carcinoma using SAGE and LongSAGE. BMC Med Genomics. 2, 5. doi: 10.1186/1755-8794-2-5.

Dong, Z., Yang, L., Lai, D., 2013. KLF5 strengthens drug resistance of ovarian cancer stem-like cells by regulating surviving expression. Cell Prolif. 46 (4), 425-435. doi: 10.1111/cpr.12043.

Eckel-Passow, J.E., Serie, D.J., Bot, B.M., Joseph, R.W., Cheville, J.C., Parker, A.S.,2014. ANKS1B is a smoking-related molecular alteration in clear cell renal cell carcinoma. BMC Urol. 14, 14. doi: 10.1186/1471-2490-14-14.

Evtimova, V., Zeillinger, R., Weidle, U.H., 2003. Identification of genes associated with the invasive status of human mammary carcinoma cell lines by transcriptional profiling. Tumour Biol. 24 (4):189-198.

Faraji, F., Pang, Y., Walker, R.C., Nieves Borges, R., Yang, L., Hunter, K.W., 2012. Cadm1 is a metástasis susceptibility gene that suppresses metástasis by modifying tumor interaction with thecell-mediated immunity. PLoS Genet. 8 (9), e1002926. doi: 10.1371/journal.pgen.1002926.

Fay, M.J., Longo, K.A., Karathanasis, G.A., Shope, D.M., Mandernach, C.J., Leong J.R., Hicks, A., Pherson, K., Husain, A., 2003. Analysis of CUL-5 expression in breast epithelial cells, breast cancer cell lines, normal tissues and tumor tissues. Mol Cancer. 2, 40. doi: 10.1186/1476-4598-2-40.

Fountzilas, E., Kotoula, V., Angouridakis, N., Karasmanis, I., Wirtz, R.M., Eleftheraki, A.G., Veltrup, E., Markou, K., Nikolaou, A., Pectasides, D., Fountzilas, G., 2013. Identification and validation of a multigene predictor of recurrence in primary laryngeal cancer. PLoS One. 8 (8), e70429. doi: 10.1371/journal.pone.0070429.

García-Pravia, C., Galván, J.A., Gutiérrez-Corral, N., Solar-García, L., García-Pérez, E., García-Ocaña, M., Del Amo-Iribarren, J., Menéndez-Rodríguez, P., García-García, J.,de Los Toyos, J.R., Simón-Buela, L., Barneo, L., 2013. Overexpression of COL11A1 by cancer-associated fibroblasts: clinical relevance of a stromal marker in pancreatic cancer. PLoS One. 8 (10), e78327. doi: 10.1371/journal.pone.0078327.

Ge, F., Chen, W., Yang, R., Zhou, Z., Chang, N., Chen, C., Zou, T., Liu, R., Tan, J., Ren, G., 2014. WWOX suppresses KLF5 expression and breast cáncer cell growth. Chin J Cancer Res. 26 (5):511-516. doi: 10.3978/j.issn.1000-9604.2014.09.03.

Hannafon, B.N., Sebastiani, P., de las Morenas, A., Lu, J., Rosenberg, C.L., 2011. Expressionof microRNA and their gene targets are dysregulated in preinvasive breast cancer. Breast Cancer Res. 13 (2), R24. doi: 10.1186/bcr2839.

Huilin, Z., Jinrong, Q., Chunping ,Y., Dazhen, Y., Lingjuan, G., Yiping, S., Xiaojun, T., Ning, X., 2014. ROR1 expression correlated with poor clinical outcome in human ovarian cáncer. Sci Rep. 4, 5811. doi: 10.1038/srep05811.

Jilaveanu, L.B., Sznol, J., Aziz, S.A., Duchen, D., Kluger, H.M., Camp, R.L., 2012. CD70 expression patterns in renal cell carcinoma. Hum Pathol. 43 (9), 1394-1399. doi: 10.1016/j.humpath.2011.10.014.

Jovov, B., Araujo-Perez, F., Sigel, C.S., Stratford, J.K., McCoy, A.N., Yeh, J.J., Keku, T., 2012. Differential gene expression between African American and European American colorectal cáncer patients. PLoS One. 7 (1), e30168. doi: 10.1371/journal.pone.0030168.

Jung, S., Sielker, S., Purcz, N., Sproll, C., Acil, Y., Kleinheinz, J., 2015. Analysis of angiogenic markers in oral squamous cell carcinoma-gene and protein expression. Head Face Med. 11, 19. doi: 10.1186/s13005-015-0076-7.

Klahan, S., Wu, M.S., Hsi, E., Huang, C.C., Hou, M.F., Chang, W.C., 2014. Computational analysis of mRNA expression profiles identifies the ITG family and PIK3R3 as crucial genes forregulating triple negative breast cáncer cell migration. Biomed Res Int. 2014, 536591. doi: 10.1155/2014/536591.

Klemke, M., Müller, M.H., Wosniok, W., Markowski, D.N., Nimzyk, R., Helmke, B.M., Bullerdiek, J., 2014. Correlated expression of HMGA2 and PLAG1 in thyroid tumors, uterine leiomyomas and experimental models. PLoS One. 9 (2), e88126. doi: 10.1371/journal.pone.0088126.

Kool, M.M., Galac, S., Kooistra, H. S., Mol, J. A., 2014. Expression of angiogenesis-related genes in canine cortisol-secreting adrenocortical tumors. Domest Anim Endocrinol. 47, 73-82. doi: 10.1016/j.domaniend.2013.11.004.

Kuroiwa, T., Yamamoto, N., Onda, T., Shibahara, T., 2009. Expression of the FAM5C in tongue squamous cell carcinoma. Oncol Rep. 22 (5):1005-1011. doi: 10.3892/or_00000528.

Kuzaka, B., Janiak, M., Włodarski, K.H., Radziszewski, P., Włodarski, P.K., 2015. Expression of bone morphogenetic protein-2 and -7 in urinary bladder cáncer predicts time to tumor recurrence. 11 (2), 378-384. doi: 10.5114/aoms.2014.46796.

Kwon, M.J., Min, S.K., Seo, J., Kim, D.H., Sung, C.O., Lim, M.S., Cho, J., Park, H.R., 2015. HIPK2 expression in progression of cutaneous epithelial neoplasm. Int J Dermatol. 54 (3), 347-354. doi: 10.1111/ijd.12664.

Li, K.K., Pang, J.C., Chung, N.Y., Ng, Y.L., Chan, N.H., Zhou, L., Poon, W.S., Ng, H.K., 2007. EMP3 overexpression is associated with oligodendroglial tumors retaining chromosome arms 1p and 19q. Int J Cancer. 120 (4): 947-950. doi: 10.1002/ijc.22415.

Li, L., Zhang, Y., Li, N., Feng, L., Yao, H., Zhang, R., Li, B., Li, X., Han, N., Gao, Y., Xiao, T., Wu, L., 2015. Nidogen-1: a candidate biomarker for ovarian serous cancer. Jpn J Clin Oncol. 45 (2), 176-182. doi: 10.1093/jjco/hyu187.

Li, Z.1., Fang, Z.Y., Ding, Y., Yao, W.T., Yang, Y., Zhu, Z.Q., Wang, W., Zhang, Q.X., 2012. Amplifications of NCOA3 gene in colorectal cancers in a Chinese population. World J Gastroenterol. 18 (8):855-860. doi: 10.3748/wjg.v18.i8.855.

Liu, K.L., Wu, J., Zhou, Y., Fan, J.H., 2015. Increased Sushi repeat-containing protein X-linked 2 is associated with progression of colorectal cancer. Med Oncol. 32 (4), 99. doi: 10.1007/s12032-015-0548-4.

Liu, S., Yin, F., Fan, W., Wang, S., Guo, X.R., Zhang, J.N., Tian, Z.M., Fan, M., 2012. Over-expression of BMPR-IB reduces the malignancy of glioblastoma cells by upregulation of p21 and p27Kip1.J Exp Clin Cancer Res. 31, 52. doi: 10.1186/1756-9966-31-52.

Lu, J. W., Chang, J.G., Yeh, K.T., Chen, R.M., Tsai, J.J., Hu, R.M., 2011. Overexpression of Thy1/CD90 in human hepatocellular carcinoma is associated with HBV infection and poor prognosis. Acta Histochem. 113 (8), 833-838. doi: 10.1016/j.acthis.2011.01.001.

Lundgren, K., Tobin, N.P., Lehn, S., Stål, O., Rydén, L., Jirström, K., Landberg, G., 2011. Stromal expression of β-arrestin-1 predicts clinical outcome and tamoxifen response in breast cancer. J Mol Diagn. 13 (3), 340-351. doi: 10.1016/j.jmoldx.2011.01.009.

Marmé, A., Zimmermann, H.P., Moldenhauer, G., Schorpp-Kistner, M., Müller, C., Keberlein, O., Giersch, A., Kretschmer, J., Seib, B., Spiess, E., Hunziker, A., Merchán F., Möller, P., Hahn, U., Kurek, R., Marmé, F., Bastert, G., Wallwiener, D., Ponstingl, H., 2008. Loss of Drop1 expression already at early tumor stages in a wide range of human carcinomas. Int J Cancer. 123 (9), 2048-2056. doi: 10.1002/ijc.23763.

Nagahara, M., Mimori, K., Kataoka, A., Ishii, H., Tanaka, F., Nakagawa, T., Sato, T., Ono, S., Sugihara, K., Mori, M., 2010. Correlated expression of CD47 and SIRPA in bone marrow and in peripheral blood predicts recurrence in breast cáncer patients. Clin Cancer Res. 16 (18), 4625-4635. doi: 10.1158/1078-0432.CCR-10-0349.

Nan, H., Xu, M., Zhang, J., Zhang, M., Kraft, P., Qureshi, A.A., Chen, C., Guo, Q., Hu, F.B., Rimm, E.B., Curhan, G., Song, Y., Amos, C.I., Wang, L.E., Lee, J.E., Wei, Q., Hunter, D.J., Han, J., 2011. Genome-wide association study identifies nidogen 1 (NID1) as susceptibility locus to cutaneous nevi and melanoma risk. Hum Mol Genet. 20 (13), 2673-2679. doi: 10.1093/hmg/ddr154.

Ohgino, K., Soejima, K., Yasuda, H., Hayashi, Y., Hamamoto, J., Naoki, K., Arai, D., Ishioka, K., Sato, T., Terai, H., Ikemura, S., Yoda, S., Tani, T., Kuroda, A., Betsuyaku, T., 2014. Expression of fibroblast growth factor 9 is associated with poor prognosis in patients with resected non-small cell lung cancer. Lung Cancer. 83 (1):90-96. doi: 10.1016/j.lungcan.2013.10.016.

Okudela, K., Mitsui, H., Suzuki, T., Woo, T., Tateishi, Y., Umeda, S., Saito, Y., Tajiri, M., Masuda, M., Ohashi, K,. 2013. Expression of HDAC9 in lung cancer—potential role in lung carcinogenesis. Int J Clin Exp Pathol. 7 (1), 213-220.

Overmeer, R.M., Henken, F.E., Snijders, P.J., Claassen-Kramer, D., Berkhof, J., Helmerhorst, T.J., Heideman, D.A., Wilting, S.M., Murakami, Y., Ito, A., Meijer, C.J., Steenbergen, R.D., 2008. Association between dense CADM1 promoter methylation and reduced protein expression in high-grade CIN and cervical SCC. J Pathol. 215 (4), 388-397. doi: 10.1002/path.2367.

Papalas, J.A., Vollmer, R.T., Gonzalez-Gronow, M., Pizzo, S.V., Burchette, J., Youens, K.E., Johnson, K.B., Selim, M.A., 2010. Patterns of GRP78 and MTJ1 expression in primary cutaneous malignant melanoma. Mod Pathol. 23 (1):134-143. doi: 10.1038/modpathol.2009.152.

Pejovic, T., Pande, N.T., Mori, M., Mhawech-Fauceglia, P., Harrington, C., Mongoue-Tchokote, S., Dim, D., Andrews, C., Beck, A., Tarumi, Y., Djilas, J., Cappuccini, F., Caballero, O., Huang, J., Levy, S., Tsiamouri, A., Cain, J., Bagby, G.C., Strausberg, R.L., Simpson, A.J., Odunsi, K.O., 2009. Expression profiling of the ovarian surface kinome reveals candidate genes for early neoplastic changes. Transl Oncol. 2 (4), 341-349.

Penney, K.L., Sinnott, J.A., Tyekucheva, S., Gerke, T., Shui, I.M., Kraft, P., Sesso H.D., Freedman, M.L., Loda, M., Mucci, L.A., Stampfer, M.J., 2015.Association of prostate cáncer risk variants with gene expression in normal and tumor tissue. Cancer Epidemiol Biomarkers Prev. 24 (1), 255-260. doi: 10.1158/1055-9965.EPI-14-0694-T.

Planeix, F., Siraj, M.A., Bidard, F.C., Robin, B., Pichon, C., Sastre-Garau, X., Antoine, M., Ghinea, N., 2015. Endothelial follicle-stimulating hormone receptor expression in invasive breast cáncer and vascular remodeling at tumor periphery. J Exp Clin Cancer Res. 34,12. doi: 10.1186/s13046-015-0128-7.

Renner, M., Goeppert, B., Siraj, M.A., Radu, A., Penzel, R., Wardelmann, E., Lehner, B., Ulrich, A., Stenzinger, A., Warth, A., Vogel, M.N., Weichert, W., Schirmacher, P., Mechtersheimer, G., Ghinea, N., 2013. Follicle-stimulating hormone receptor expression in soft tissue sarcomas. Histopathology. 63 (1):29-35. doi: 10.1111/his.12135.

Rink, L., Skorobogatko, Y., Kossenkov, A.V., Belinsky, M.G., Pajak, T., Heinrich, M.C., Blanke, C.D., von Mehren, M., Ochs, M.F., Eisenberg, B., Godwin, A.K., 2009. Gene expression signatures and response to imatinib mesylate in gastrointestinal stromal tumor.Mol Cancer Ther. 8 (8):2172-2182. doi: 10.1158/1535-7163.MCT-09-0193.

Robert, N.M., Martin, L.J., Tremblay, J.J., 2006. The orphan nuclear receptor NR4A1 regulates insulin-like 3 gene transcription in Leydig cells. Biol Reprod. 74 (2), 322-330. doi: 10.1095/biolreprod.105.044560.

Savinainen,,K.J., Linja, M.J., Saramäki, O.R., Tammela, T.L., Chang, G.T., Brinkmann, A.O, Visakorpi, T., 2004. Expression and copy number analysis of TRPS1, EIF3S3 and MYC genes in breast and prostate cancer.Br J Cancer. 90(5):1041-6.

Scaggiante, B., Dapas, B., Bonin, S., Grassi, M., Zennaro, C., Farra, R., Cristiano, L., Siracusano, S., Zanconati, F., Giansante, C., Grassi, G., 2012. Dissecting the expression of EEF1A1/2 genes in human prostate cáncer cells: the potential of EEF1A2 as a hallmark for prostate transformation and progression. Br J Cancer.106 (1):166-173. doi: 10.1038/bjc.2011.500.

Scrideli, C.A., Carlotti, C.G.Jr., Okamoto, O.K., Andrade, V.S., Cortez, M.A., Motta, F.J., Lucio-Eterovic, A.K,, Neder, L., Rosemberg, S., Oba-Shinjo, S.M., Marie, S.K., Tone, L.G., 2008. Gene expression profile analysis of primary glioblastomas and non-neoplastic brain tissue: identification of potential target genes by oligonucleotide microarray and real-time quantitative PCR. J Neurooncol. 88 (3), 281-291. doi: 10.1007/s11060-008-9579-4.

Segditsas, S., Sieber, O., Deheragoda, M., East, P., Rowan, A., Jeffery, R., Nye, E., Clark S., Spencer-Dene, B., Stamp, G., Poulsom, R., Suraweera, N., Silver, A., Ilyas, M., Tomlinson, I., 2008. Putative direct and indirect Wnt targets identified through consistent gene expression changes in APC-mutant intestinal adenomas from humans and mice. Hum Mol Genet. 17 (24), 3864-3875. doi: 10.1093/hmg/ddn286.

Shimazui, T., Yoshikawa, K., Uemura, H., Hirao, Y., Saga, S., Akaza, H., 2004. The level of cadherin-6 mRNA in peripheral blood is associated with the site of metástasis

and with thesubsequent occurrence of metastases in renal cell carcinoma. Cancer. 101 (5), 963-968. doi: 10.1002/cncr.20479.

Sivadas, V.P., Saakshi, G., Iype, E.M., Balan, A., Kannan, S., 2015. Prognostic implication of the loss of TGFBR2 expression in oral carcinoma. Neoplasma. 62 (3), 398-404.

Sørensen, K.D., Abildgaard, M.O., Haldrup, C., Ulhøi, B.P., Kristensen, H., Strand, S., Parker, C., Høyer, S., Borre, M., Ørntoft, T.F., 2013. Prognostic significance of aberrantly silenced ANPEP expression in prostate cancer. Br J Cancer. 108 (2), 420-428. doi: 10.1038/bjc.2012.549.

Stokes, A., Joutsa, J., Ala-Aho, R., Pitchers, M., Pennington, C.J., Martin, C., Premachandra, D.J., Okada, Y., Peltonen, J., Grénman, R., James, H. A., Edwards, D.R., Kähäri, V.M., 2010. Expression profiles and clinical correlations of degradome components in the tumor microenvironment of head and neck squamous cell carcinoma. Clin Cancer Res.16 (7), 2022-2035. doi: 10.1158/1078-0432.CCR-09-2525.

Sugihara, H., Ishimoto, T., Watanabe, M.., Sawayama, H., Iwatsuki, M.., Baba, Y., Komohara, Y., Takeya, M.., Baba, H., 2013. Identification of miR-30e* regulation of Bmi1 expression mediated by tumor-associated macrophages in gastrointestinal cancer. PLoS One. 8 (11), e81839. doi: 10.1371/journal.pone.0081839.

Suh, I., Shibru, D., Eisenhofer, G., Pacak, K.,Duh, Q.Y., Clark, O.H., Kebebew, E., 2009. Candidate genes associated with malignant pheochromocytomas by genome-wide expression profiling.Ann Surg. 250 (6), 983-990. doi: 10.1097/SLA.0b013e3181b248bb.

Sun, B., Wu, J., Zhang, T., Wang, C., 2008. High-resolution analysis of genomic profiles of hepatocellular carcinoma cells with differential osteopontin expression. Cancer Biol Ther. 7 (3), 387-391.

Sun, G., Zhaom, G., Lu, Y., Wang, Y., Yang, C., 2014b. Association of EMP1 with gastric carcinoma invasion, survival and prognosis. Int J Oncol. 45 (3), 1091-1098. doi: 10.3892/ijo.2014.2488.

Sun, G.G., Wang, Y,D., Cui, D.W., Cheng, Y.J., Hu, W.N., 2014a. Epithelial membrane protein 1 negatively regulates cell growth and metastasis in colorectal carcinoma. World J Gastroenterol. 20 (14):4001-4010. doi: 10.3748/wjg.v20.i14.4001.

Takahashi, H, Nakatsuji, H., Takahashi, M., Avirmed, S., Fukawa, T., Takemura, M., Fukumori, T., Kanayama, H., 2012. Up-regulation of plakophilin-2 and down-regulation of plakophilin-3 are correlated with invasiveness in bladder cancer. Urology. 79 (1), 240.e1-8. doi: 10.1016/j.urology.2011.08.049.

Takahashi, H., Nemoto, T., Yoshida, T., Honda, H., Hasegawa, T., 2006. Cancer diagnosis marker extraction for soft tissue sarcomas based on gene expression profiling data by using projective adaptive resonance theory (PART) filtering method. BMC Bioinformatics. 7, 399. doi: 10.1186/1471-2105-7-399.

Tang, Q., Wu, W., Xu, X., Huang, L., Gao, Q., Chen, H., Sun, H., Xia, Y., Sha, J., Wang, X., Chen, D., Xu, Q., 2013. miR-141 contributes to fetal growth restriction by regulating PLAG1 expression. PLoS One. 8 (3), e58737. doi: 10.1371/journal.pone.0058737.

Thedieck, C., Kalbacher, H., Kuczyk, M., Müller, G.A., Müller, C.A., Klein, G., 2007. Cadherin-9 is a novel cell surface marker for the heterogeneous pool of renal fibroblasts. PLoS One. 2 (7), e657. doi: 10.1371/journal.pone.0000657

Tomlinson, V.A., Newbery, H.J., Bergmann, J.H., Boyd, J., Scott, D., Wray, N.R., Sellar, G.C., Gabra, H., Graham, A., Williams, A.R., Abbott, C.M., 2007. Expression of eEF1A2 is associated with clear cell histology in ovarían carcinomas: overexpression of the geneis not dependent on modifications at the EEF1A2 locus. Br J Cancer. 96 (10),1613-1620. doi: 10.1038/sj.bjc.6603748.

Wang, C., Yu, G., Liu, J., Wang, J., Zhang, Y., Zhang, X., Zhou, Z., Huang, Z., 2012. Downregulation of PCDH9 predicts prognosis for patients with glioma. J Clin Neurosci. 19 (4), 541-545. doi: 10.1016/j.jocn.2011.04.047.

Wiese, A.H., Auer, J., Lassmann, S., Nährig, J., Rosenberg, R., Höfler, H., Rüger, R., Werner, M., 2007. Identification of gene signatures for invasive colorectal tumor cells. Cancer. Detect Prev. 31 (4):282-295. doi:10.1016/j.cdp.2007.07.003.

Xing, Q.T., Qu, C.M., Wang, G., 2014 Overexpression of Abl2 predicts poor prognosis in hepatocellular carcinomas and is associated with cáncer cell migration and invasión. Onco Targets Ther.7, 881-885. doi: 10.2147/OTT.S62348.

Yamada, T., Oshima, T., Yoshihara, K., Sato, T., Nozaki, A., Shiozawa, M., Ota, M., Yoshikawa, T., Akaike, M., Numata, K., Rino, Y., Kunisaki, C., Tanaka, K., Imada, T., Masuda, M., 2014. Impact of overexpression of Sushi repeat-containing protein X-linked 2 gene on outcomes of gastric cancer. J Surg Oncol.109 (8),836-840. doi: 10.1002/jso.23602.

Yang, S., Lu, M., Chen, Y., Meng, D., Sun, R., Yun, D., Zhao, Z., Lu, D., Li, Y., 2015. Overexpression of eukaryotic elongation factor 1 alpha-2 is associated with

poorer prognosis in patients with gastric cancer. J Cancer Res Clin Oncol. 141 (7), 1265-1275. doi: 10.1007/s00432-014-1897-7.

Zatkova, A., Rouillard, J.M., Hartmann, W., Lamb, B.J., Kuick, R., Eckart, M., von Schweinitz, D., Koch, A., Fonatsch, C., Pietsch, T., Hanash, S.M., Wimmer, K., 2004. Amplification and overexpression of the IGF2 regulator PLAG1 in hepatoblastoma. Genes Chromosomes Cancer. 39 (2):126-137. doi: 10.1002/gcc.10307.

Zhang, J., Cao, W., Xu, Q., Chen, W.T., 2011. The expression of EMP1 is downregulated in oral squamous cell carcinoma and possibly associated with tumour metastasis. J Clin Pathol. 64 (1):25-29. doi: 10.1136/jcp.2010.082404.

Zhang, X., Zhang, R., Zheng, Y., Shen, J., Xiao, D., Li, J., Shi, X., Huang, L., Tang, H., Liu, J., He, J., Zhang, H., 2013. Expression of gamma-aminobutyric acid receptors on neoplastic growth and prediction of prognosis in non-small cell lung cancer. J Transl Med. 11, 102. doi: 10.1186/1479-5876-11-102.

Zheng, L., Pu, J., Jiang, G., Weng, M., He, J., Mei, H., Hou, X., Tong, Q., 2010. Abnormal expression of early growth response 1 in gastric cancer: association with tumor invasion, metastasis and heparanase transcription. Pathol Int. 60 (4), 268-277. doi: 10.1111/j.1440-1827.2010.02512.x.

Zubor, P., Hatok, J, Moricova, P., Kajo, K., Kapustova, I., Mendelova, A., Racay, P., Danko, J., 2015. Gene expression abnormalities in histologically normal breast epithelium from patients with luminal type ofbreast cancer. Mol Biol Rep. 42 (5), 977-988. doi: 10.1007/s11033-014-3834-x.

Discusión

La infección con los Virus del Papiloma Humano de Alto Riesgo (VPH-AR) se asocia con el desarrollo de cáncer cervical, específicamente el genotipo 16 que con mayor frecuencia se detecta tanto en cáncer cervical como en lesiones escamosas intraepiteliales de alto grado (Obeidat *et al.,* 2013, Crosbie *et al.,* 2013). El VPH 16 presenta diferentes variantes debido a polimorfismos en su secuencia de ADN, tales polimorfismos se pueden traducir a cambios de aminoácidos que generan cambios en la estructura de la oncoproteina E6, lo que podria alterar la interacción de E6 con sus proteínas blanco, de esta forma puede influir en el potencial oncogénico de E6 de las variantes del VPH 16. De manera adicional, polimorfismos presentes en los pacientes genera que el riesgo para desarrollar cáncer cervical dependa de la población en estudio. (Xi *et al.,* 2007, Cornet *et al.,* 2012, Tornesello *et al.* 2011, Pillai *et al.* 2009, Xi *et al.,* 2006). Se ha reportado que en población del sur de México las variantes del VPH 16 que presentan las más altas frecuencias y riesgo para desarrollar cáncer cervical son: AA-a, AA-c, E-G350, E-C188/G350 y E-A176/G350 (Ortiz-Ortiz *et al.,* 2015).

Por otro lado, se ha demostrado que uno de los mecanismos mediante el cual las variantes del VPH 16 pueden inducir el desarrollo de cáncer cervical es la alteración en la expresión de genes celulares que participan en el desarrollo de cáncer cervical (Jang *et al.,* 2011, Sichero *et al.,* 2012). En este estudio se generó un sistema de células C33-A que expresa establemente a E6 del las variantes AA-a, AA-c, E-G350, E-C188/G350 y E-A176/G350 del VPH 16, con el que se demostró que la expresión de E6 de E-Prototipo altera la expresión de genes que participan en el desarrollo de cáncer, sin embargo, la expresión de E6 de las variantes del VPH 16 induce la alteración en la expresión de genes de manera diferencial a los genes que alteran su expresión por la E-Prototipo, la expresión de cada E6 de las variantes del VPH 16 genera un patrón diferencial en la expresión de genes. Otros estudios han analizado la alteración en la expresión de genes cuando

se expresa a E6 de las variantes AA-a y E-G350 (Jang *et al*., 2011, Sichero *et al.,* 2012, Richard *et al.,* 2010, Lichtig *et al.,* 2006), sin embargo, no hay estudios que analicen la expresión de genes cuando se expresa a E6 de las variantes AA-c, E-C188/G350 y E-A176/G350.

En este estudio se analizó la expresión del transcriptoma completo de células C33-A cuando expresan E6 de las variantes del VPH 16 y E-prototipo. Se observó que la variante E-A176/G350 expresa una mayor cantidad de E6 en comparación con las otras variantes de estudio (Fig 1), no hay estudios *in vitro* hasta el momento sobre el potencial oncogénico de E-A176/G350 debido a que en otras poblaciones no representa una alta frecuencia en cáncer cervical, sin embargo, el presente estudio sugiere que el alto riesgo para desarrollar cáncer cervical podria estar relacionado a la alta expresión de la oncoproteina E6. Los resultados obtenidos *in silico* por Pillai *et al.* (2009) determinaron que el polimorfismo presente en el nucleótido 176 afecta la interacción de E6 con el epítope de celulas T, lo cual podria generar una inhibición de la respuesta inmune contra E6, las pacientes infectadas con la variante E-A176/G350 tienen un riesgo del 39.82 veces más de desarrollar cáncer que cuando la infección es por E-prototipo de acuerdo con los estudios de Ortiz-Ortiz *et al.* (2015).

La expresión de E6 de E-Prototipo alteró la expresión de 266 genes vs. mock (Fig 2). Jang *et al.* (2011), al igual que que en este estudio, transfectaron a células C33-A con E6 de E-Prototipo, obteniendo como resultado la alteración de 195 genes cuando se expresa E6 de E-ptototipo vs. Mock de los cuales 2 genes coinciden con el presente estudio (FBXO5 y TXNRD1). La diferencia en la expresión de genes puede deberse a que la plataforma de microarreglos utilizada por Jang *et al.* (2011), y este estudio son diferentes, además de que ellos utilizaron al plásmido pLenti6.3/V5-TOPO y en este estudio se utilizó al plásmido pEGFPN1, lo que pudo alterar la expresión de genes de manera diferencial en C33-A.

En este estudio se observó que la expresión del gen E6 puede alterar la expresión de genes pero que la alteración en su expresión puede ser

diferente dependiendo de si se expresa E6 de las variantes del VPH 16 o E6 de E-Prototipo, de los 44 genes que alteran su expresión de manera compartida cuando se expresa E6 de E-Prototipo y E6 de variantes del VPH 16, 32 genes son regulados de manera inversa entre las variantes y la E-Prototipo (Fig 2 y tabla S1). Por ejemplo, el gen CUBN aumentada su expresión por E6 de la E-Prototipo, mientras que su expresión está disminuida por E6 de las variantes AA-a, AA-c, E-C188/G350 y E-G350. Un aumento en la expresión de FMN2 promueve el arresto del ciclo ciclo celular por la inhibición de p21 (Yamada *et al.,* 2013a, Yamada *et al.,* 2013b), en nuestros resultados FMN2 aumenta su expresión por al expresión de E-Prototipo, sin embargo, cuando se expresa E6 de las variantes, la expresión de este gen dismunuye, por lo anterior E6 de E-Prototipo podria inducir arresto del ciclo celular mientras que cuando se expresa E6 de las variantes del VPH 16 se podria inducir progresión del ciclo celular. Se suguiere que el potencial oncogénico de las variantes del VPH 16 no solomente depende de los genes que alteran diferencialmente en comparación con E-Prototipo sino de la expresión del conjunto de genes cuado se expresa E6 de las variantes del VPH 16.

Por otro lado, se analizaron a los genes que alteran su expresión de manera compartida entre las variantes del VPH 16, excluyendo a los genes que alteran su expresión por E-prototipo (Fig S1 y Tabla S2). Se observa que la variante AA-a no comparte ningún gen con las variantes E-A176/G350 y E-C188/G350, además de que las variantes E-A176/G350 vs. E-C188/G350 son las que comparten el mayor número de genes (Fig. S1, Tabla S2). Estos resultados podrían deberse a que la variante AA-a en comparación con las variantes E-A176/G350 y E-C188/G350 pertenecen a ramas filogenéticas diferentes, sin embargo, las variantes E-A176/G3350 y E-C188/G350 pertenecen a la misma rama filogenética.

Identificamos que las variantes AA-c y E-G350 son las que alteran un mayor número de genes (Fig 2), la variante AA-a es la variante más parecida a la E-prototipo debido a que de los 37 genes que alteran su expresión por la

expresion de E6, 15 genes son compartidos con E-Prototipo (Fig 2). Los resultados de este estudio sugieren que el potencial oncogénico de las variantes de E6 no está dado por el número de genes alterados, se observó que la variante AA-a altera la expresión de 22 genes, los cuales son pocos genes en comparación con el numero de genes alterados por la expresión de E6 de la variante AA-c, sin embargo, se ha demostrado que el potencial oncogénico de la AA-a es mayor que de la AA-c (Ortiz-Ortiz *et al.,* 2015). Varios estudios han analizado la expresión de genes en células transfectadas con E6 de la variante AA-a y los resultados obtenidos los han generalizado para todas las variantes de AA (Sichero *et al.,* 2012, Richard *et al.,* 2012, Lichtig *et al.,* 2006), sin embargo, nuestros resultados nos permiten demostrar que existe una expresión diferencial de genes cuando se expresa E6 de las variantes AA-a y AA-c y esto puede influir en el desarrollo de cáncer cervical.

Categorizamos a los genes alterados por la expresión de E6 de las variantes vs. E-Prototipo de acuerdo al proceso celular en el cual participan. Se sugiere que las variantes de E6 podrían presentar mayor potencial oncogénico en comparación con E6 de E-Prototipo debido a que alteran la expresión de genes que participan en procesos que conducen a cáncer. Este estudio demuestra que la mayoría de los genes que alteran su expresión cuando se expresa E6 de las variantes vs. E-Prototipo están involucrados en señalización celular (20.3%), transcripción (16.7%) y adhesión (14.6%), sin embargo, no solamente se debe considerar el número de genes involucrados en un proceso celular para analizar el potencial oncogénico de las variantes, se debe considerar al conjunto de genes que desregulan o sobreregulan su expresión en celulas C33-A cuando se expresa E6 de las variantes. Para analizar este aspecto se realizó un análisis de "*enrichmet score*" (Fig 5 y Suplemento S4), en el que se observa que la expresión de E6 de la variante AA-a altera la expresión de genes asociados con adhesión celular y via de señalización tirosina cinasa. Estos resultados se relacionan con lo obtenido por Niccoli *et al.* (2012), debido a que demostró que los queratinocitos de prepucio humano primario presentan una alta capacidad migratoria cuando

expresan E6 de la variante AA-a, la migración es un proceso tardio de la desregulación de la adhesión.

Todas las variantes estudiadas presentan el cambio de aminoácido L83V en el dominio carboxilo terminal en comparación con la E6 de E-Prototipo, sin embargo, las variantes estudiadas presentan cambios adicionales de aminoácidos en el extremo amino terminal, los cuales las hacen diferentes entre ellas. De la rama AA, la variante AA-a presenta 3 cambios de aminoácidos (Q14H/H78Y/L83V) y la variante AA-c 4 cambios de aminoácidos (Q14H/I27R/H78Y/L83V); se sugiere que el aminoácido 27 es importante para que E6 lleve a cabo su función, este cambio de aminoácido podria estar implicado en que E6 de la variante AA-a y AA-c presenten diferente potencial oncogénico. Lo mismo ocurre para el caso de las variantes europeas, se puede observar que los cambios de aminoácidos D25N y E29Q presentes en las variantes E-A176/G350 y E-C188/G350, respectivamente, inducen que la proteina E6 module de manera diferente la expresión de genes que cuando solo se encuentra el cambio del aminoácido L83V.

En conclusión, la expresión de E6 de cada una de las variantes del VPH 16 induce un patrón de expresión del transcriptoma humano diferente. Cada variante parece tener su propia firma molecular, la cual puede conferir ventajas en algunas funciones pero limitación en otras. Los genes que alteran su expresión bajo la expresion de E6 de las variantes del VPH 16 participan en procesos que se relacionan con el desarrollo de cáncer cervical, lo que puede contribuir a explicar la diferencia de oncogenicidad entre las variantes del VPH 16 y la E-Prototipo. Adicionalmente, no se identificó un conjunto de genes que alteren su expresión por variantes de la misma rama filogenética.

Perspectivas

Para anlizar el potencial oncogenico de las variantes del VPH 16 y determinar el mecanismo mediante el cual inducen el desarrollo de cáncer, faltan varios puntos por estudiar.

- Analizar los niveles de proteínas de los genes que presentaron alteración en su expresión a nivel de mRNA.
- Realizar análisis *in silico* para determinar si los cambios de aminoácidos presentes en E6 de las variantes del VPH 16 inducen cambios en su estructura.
- Determinar si los polimorfimos presentes en E6 de las variantes afectan la interacción entre E6 y sus proteínas blanco.
- Evaluar *in vitro* los procesos celulares que de acuerdo al análisis de enrichment score podrían estar alterados cuando se expresan E6 de las variantes de estudio.

Referencias

Bhattacharjee B, Mandal NR, Roy S, Sengupta S. 2008. Characterization of sequence variations within HPV16 isolates among Indian women: prediction of causal role of rare non-synonymous variations within intact isolates in cervical cancer pathogenesis.Virology. 377(1):143-50. doi: 10.1016/j.virol.2008.04.007. Epub 2008 May 20.

Chen JJ, Reid CE, Band V, Androphy EJ. 1995. Interaction of papillomavirus E6 oncoproteins with a putative calcium-binding protein. Science. 269(5223):529-31.

Cornet, I., Gheit, T., Franceschi, S., Vignat, J., Burk, R.D., Sylla, B.S., Tommasino, M., Clifford, G.M., 2012. Human papillomavirus type 16 genetic variants: phylogeny and classification based on E6 and LCR. J Virol. 86 (12), 6855-6861. doi: 10.1128/JVI.00483-12.

Crosbie, E.J., Einstein, M.H., Franceschi, S., Kitchener, H. C., 2013. Human papillomavirus and cervical cancer. Lancet. 382 (9895), 889-899. doi: 10.1016/S0140-6736(13)60022-7.

de Araujo Souza PS, Maciag PC, Ribeiro KB, Petzl-Erler ML, Franco EL, Villa LL. 2008. Interaction between polymorphisms of the human leukocyte antigen and HPV-16 variants on the risk of invasivecervical cancer. BMC Cancer. 8:246. doi: 10.1186/1471-2407-8-246.

de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H, 2004. Classification of papillomaviruses. Virology. 324(1):17-27.

Degenhardt YY Silverstein S. 2001. Interaction of zyxin, a focal adhesion protein, with the e6 protein from human papillomavirus type 6 results in its nuclear translocation. J Virol. 2001 Dec;75(23):11791-802.

Doorbar J, 2005. The papillomavirus life cycle. J Clin Virol. 32 Suppl 1:S7-15.

Doorbar J, 2007. Papillomavirus life cycle organization and biomarker selection. Dis Markers. 23(4):297-313.

Ennaifer E, Salhi F, Laassili T, Fehri E, Alaya NB, Guizani I, Boubaker S, 2015. Type-Specific Human Papillomavirus Distribution in Invasive

Squamous Cervical Carcinomas in Tunisia and Vaccine Impact. Asian Pac J Cancer Prev. 16(15):6769-72.

Gao Q, Srinivasan S, Boyer SN, Wazer DE, Band V, 1999. The E6 oncoproteins of high-risk papillomaviruses bind to a novel putative GAP protein, E6TP1, and target it for degradation. Mol Cell Biol. 19(1):733-44.

Gao Q, Singh L, Kumar A, Srinivasan S, Wazer DE, Band V, 2001. Human papillomavirus type 16 E6-induced degradation of E6TP1 correlates with its ability to immortalize humanmammary epithelial cells. J Virol. 75(9):4459-66.

Garnett TO, Duerksen-Hughes PJ. 2006. Modulation of apoptosis by human papillomavirus (HPV) oncoproteins. Arch Virol. 151(12):2321-35. Epub 2006 Jul 27.

Gewin L, Myers H, Kiyono T, Galloway DA, 2004. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex. Genes Dev. 18(18):2269-82.

Glaunsinger BA, Lee SS, Thomas M, Banks L, Javier R. 2000. Interactions of the PDZ-protein MAGI-1 with adenovirus E4-ORF1 and high-risk papillomavirus E6 oncoproteins. Oncogene. 19(46):5270-80.

Huertas-Salgado, A., Martín-Gámez, D.C., Moreno, P., Murillo, R., Bravo, M.M., Villa, L., Molano, M., 2011. E6 molecular variants of human papillomavirus (HPV) type 16: an updated and unified criterion for clustering and nomenclature. Virology. 410 (1), 201-215. doi: 10.1016/j.virol.2010.10.039.

Iftner T, Elbel M, Schopp B, Hiller T, Loizou JI, Caldecott KW, Stubenrauch F. 2002. Interference of papillomavirus E6 protein with single-strand break repair by interaction with XRCC1. EMBO J. 21(17):4741-8.

Jang, M., Rhee, J.E., Jang, D.H., Kim, S.S., 2011. Gene expression profiles are altered in human papillomavirus-16 E6 D25E-expressing cell lines. Virol J. 8, 453. doi: 10.1186/1743-422X-8-453.

Jeong KW, Kim HZ, Kim S, Kim YS, Choe J. 2007. Human papillomavirus type 16 E6 protein interacts with cystic fibrosis transmembrane regulator-associatedligand and promotes E6-associated protein-mediated ubiquitination and proteasomal degradation. Oncogene. 26(4):487-99. Epub 2006 Jul 31.

Jiang M, Baseman JG, Koutsky LA, Feng Q, Mao C, Kiviat NB, Xi LF., 2009. Sequence variation of human papillomavirus type 16 and measurement of viral integration by quantitative PCR. J Clin Microbiol. 47(3):521-6. doi: 10.1128/JCM.02115-08. Epub 2008 Dec 30.

Jing M, Bohl J, Brimer N, Kinter M, Vande Pol SB, 2007. Degradation of tyrosine phosphatase PTPN3 (PTPH1) by association with oncogenic human papillomavirus E6proteins. J Virol. 81(5):2231-9. Epub 2006 Dec 13.

Kiyono T, Hiraiwa A, Fujita M, Hayashi Y, Akiyama T, Ishibashi M, 1997. Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the Drosophila discs large tumor suppressor protein..Proc Natl Acad Sci U S A. 94(21):11612-6.

Koskimaa HM, Kurvinen K, Costa S, Syrjänen K, Syrjänen S. 2010. Molecular markers implicating early malignant events in cervical carcinogenesis. Cancer Epidemiol Biomarkers Prev. 19(8):2003-12. doi: 10.1158/1055-9965.EPI-09-0781. Epub 2010 Jul 20.

Kukimoto I, Aihara S, Yoshiike K, Kanda T, 1998. Human papillomavirus oncoprotein E6 binds to the C-terminal region of human minichromosome maintenance 7 protein. Biochem Biophys Res Commun. 249(1):258-62.

Lazarczyk M, Cassonnet P, Pons C, Jacob Y, Favre M., 2009. The EVER proteins as a natural barrier against papillomaviruses: a new insight into the pathogenesis of human papillomavirus infections. Microbiol Mol Biol Rev. Jun;73 (2): 348-370. doi: 10.1128/MMBR.00033-08.

Lee C, Laimins LA, 2004. Role of the PDZ domain-binding motif of the oncoprotein E6 in the pathogenesis of human papillomavirus type 31. J Virol. 78(22):12366-77.

Lee SS, Glaunsinger B, Mantovani F, Banks L, Javier RT. 2000. Multi-PDZ domain protein MUPP1 is a cellular target for both adenovirus E4-ORF1 and high-risk papillomavirus type 18 E6 oncoproteins. J Virol. 74(20):9680-93.

Lichtig, H., Algrisi, M., Botzer, L.E., Abadi, T., Verbitzky, Y., Jackman, A., Tommasino, M., Zehbe, I., Sherman, L., 2006. HPV16 E6 natural variants exhibit different activities in functional assays relevant to the carcinogenic potential of E6. Virology. 350 (1), 216-227. doi: 10.1016/j.virol.2006.01.038.

Liu Y, Chen JJ, Gao Q, Dalal S, Hong Y, Mansur CP, Band V, Androphy EJ. 1999. Multiple functions of human papillomavirus type 16 E6 contribute to the immortalization of mammary epithelial cells. J Virol. 73(9):7297-307.

liuardt YY, Silverstein SJ, 2001.Gps2, a protein partner for human papillomavirus E6 proteins.J Virol. 75 (1):151-60.

Lu Z, Hu X, Li Y, Zheng L, Zhou Y, Jiang H, Ning T, Basang Z, Zhang C, Ke Y. 2004. Human papillomavirus 16 E6 oncoprotein interferences with insulin signaling pathway by binding to tuberin. J Biol Chem. 279(34):35664-70. Epub 2004 Jun 1.

Mantovani F, Banks L.2001. The human papillomavirus E6 protein and its contribution to malignant progression. Oncogene. 20(54):7874-87.

Mattiussi S, Matsumoto K, Illi B, Martelli F, Capogrossi MC, Gaetano C. 2006. Papilloma protein E6 abrogates shear stress-dependent survival in human endothelial cells: evidence for specialized functions of paxillin. Cardiovasc Res. 70(3):578-88. Epub 2006 Feb 28.

Mischo A, Ohlenschläger O, Hortschansky P, Ramachandran R, Görlach M, 2013. Structural insights into a wildtype domain of the oncoprotein E6 and its interaction with a PDZ domain. PLoS One. 8(4):e62584. doi: 10.1371/journal.pone.0062584. Print 2013.

Molijn A, Jenkins D1, Chen W2, Zhang X2, Pirog E3, Enqi W4, Liu B2, Schmidt J5, Cui J2, Qiao Y2, Quint W1; Chinese HPV Typing Group. 2015.

The complex relationship between human papillomavirus and cervical adenoc arcinoma. Int J Cancer. 138 (2), 409-416. doi: 10.1002/ijc.29722

Muñoz N, Castellsagué X, Berrington de González A, Gissmann L, 2006. HPV in the etiology of human cancer, Vaccine. 24 (3), S1-S10

Muschik D, Braspenning-Wesch I, Stockfleth E, Rösl F, Hofmann TG, Nindl I, 2011. Cutaneous HPV23 E6 prevents p53 phosphorylation through interaction with HIPK2. PLoS One. 6(11):e27655. doi: 10.1371/journal.pone.0027655. Epub 2011 Nov 16.

Narisawa-Saito M, Kiyono T. 2007. Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and E7 proteins. Cancer Sci. 98(10):1505-11. Epub 2007 Jul 23.

Nguyen MM, Nguyen ML, Caruana G, Bernstein A, Lambert PF, Griep AE. 2003. Requirement of PDZ-containing proteins for cell cycle regulation and differentiation in the mouse lens epithelium. Mol Cell Biol. 23(24):8970-81.

Niccoli, S., Abraham, S., Richard, C., Zehbe, I., 2012. The Asian-American E6 variant protein of human papillomavirus 16 alone is sufficient to promoteimmortalization, transformation, and migration of primary human foreskin keratinocytes. J Virol. 86 (22), 12384-12396. doi: 10.1128/JVI.01512-12.

Nominé Y, Masson M, Charbonnier S, Zanier K, Ristriani T, Deryckère F, Sibler AP, Desplancq D, Atkinson RA, Weiss E, Orfanoudakis G, Kieffer B, Travé G, 2006. Structural and functional analysis of E6 oncoprotein: insights in the molecular pathways of human papillomavirus-mediated pathogenesis. Mol Cell. 21(5):665-78.

Obeidat, B., Matalka, I., Mohtaseb, A., Jaradat, S., Hayajneh, W., Khasawneh, R., Haddad H., Obeidat, F., 2013. Prevalence and distribution of high-risk human papillomavirus genotypes in cervical carcinoma, low-grade, and high-grade squamous intraepithelial lesions in Jordanian women. Eur J Gynaecol Oncol. 34 (3), 257-260.

Oliver AW1, He X, Borthwick K, Donne AJ, Hampson L, Hampson IN. 2011. The HPV16 E6 binding protein Tip-1 interacts with ARHGEF16, which

activates Cdc42. Br J Cancer.104(2):324-31. doi: 10.1038/sj.bjc.6606026. Epub 2010 Dec 7.

Ortiz-Ortiz, J., Alarcón-Romero, L del C., Jiménez-López, M.A., Garzón-Barrientos, V.H., Calleja-Macías, I., Barrera-Saldaña, H-A., Leyva-Vázquez, M.A., Illades-Aguiar, B., 2015. Association of human papillomavirus 16 E6 variants with cervical carcinoma and precursor lesions in women from Southern Mexico. Virol J. 12, 29. doi: 10.1186/s12985-015-0242-3.

Park JS, Kim EJ, Lee JY, Sin HS, Namkoong SE, Um SJ, 2001. Functional inactivation of p73, a homolog of p53 tumor suppressor protein, by human papillomavirus E6 proteins. Int J Cancer. 91(6):822-7.

Patel D, Huang SM, Baglia LA, Mc Cance DJ. 1999. The E6 protein of human papillomavirus type 16 binds to and inhibits co-activation by CBP and p300. EMBO J. 18(18):5061-72.

Pillai, M.R., Hariharan, R., Babu, J.M., Lakshmi, S., Chiplunkar, S.V., Patkar M., Tongaonkar, H., Dinshaw, K., Jayshree, R.S., Reddy, B.K., Siddiqui, M., Roychoudury, S., Saha, B., Abraham, P., Gnanamony, M., Peedicayil, A., Subhashini, J., Ram, T.S., Dey, B., Sharma, C., Jain, S.K., Singh, N., 2009. Molecular variants of HPV-16 associated with cervical cancer in Indian population. Int J Cancer. 125 (1), 91-103. doi: 10.1002/ijc.24322.

Richard, C., Lanner, C., Naryzhny, S.N., Sherman, L., Lee, H., Lambert, P.F., Zehbe, I., 2010. The immortalizing and transforming ability of two common human papillomavirus 16 E6 variants with different prevalences in cervical cancer. Oncogene. 29 (23), 3435-3445. doi: 10.1038/onc.2010.93.

Ristriani T, Masson M, Nominé Y, Laurent C, Lefevre JF, Weiss E, Travé G. 2000.HPV oncoprotein E6 is a structure-dependent DNA-binding protein that recognizes four-way junctions. J Mol Biol. 296(5):1189-203.

Ronco LV, Karpova AY, Vidal M, Howley PM. 1998. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev. 12(13):2061-72.

Sakakibara N, Mitra R, McBride AA. 2011. The papillomavirus E1 helicase activates a cellular DNA damage response in viral replication foci. J Virol. 85(17):8981-95. doi: 10.1128/JVI.00541-11. Epub 2011 Jul 6.

Sichero, L., Sobrinho, J.S., Villa, L.L., 2012. Oncogenic potential diverge among human papillomavirus type 16 natural variants. Virology. 432 (1), 127-132. doi: 10.1016/j.virol.2012.06.011.

Suprynowicz FA, Krawczyk E, Hebert JD, Sudarshan SR, Simic V, Kamonjoh CM, Schlegel R. 2010 The human papillomavirus type 16 E5 oncoprotein inhibits epidermal growth factor trafficking independently of endosome acidification. J Virol. 84(20):10619-29. doi: 10.1128/JVI.00831-10. Epub 2010 Aug 4.

Theelen W, Reijans M, Simons G, Ramaekers FC, Speel EJ, Hopman AH., 2010. A new multiparameter assay to assess HPV 16/18, viral load and physical status together with gain of telomerase genes in HPV-related cancers. Int J Cancer. 126(4):959-75. doi: 10.1002/ijc.24844.

Thomas M, Banks L.1999. Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types. J Gen Virol. 80 (Pt 6):1513-7.

Tornesello, M.L., Losito, S., Benincasa, G., Fulciniti, F., Botti, G., Greggi, S., Buonaguro, L., Buonaguro, F.M., 2011. Human papillomavirus (HPV) genotypes and HPV16 variants and risk of adenocarcinoma and squamous cell carcinoma of the cervix. Gynecol Oncol. 121 (1), 32-42. doi: 10.1016/j.ygyno.2010.12.005.

Veldman T, Liu X, Yuan H, Schlegel R, 2003. Human papillomavirus E6 and Myc proteins associate in vivo and bind to and cooperatively activate the telomerase reverse transcriptase promoter. Proc Natl Acad Sci U S A. 2003 Jul 8;100(14):8211-6. Epub 2003 Jun 23.

Wise-Draper TM1, Wells SI. 2008. Papillomavirus E6 and E7 proteins and their cellular targets. Front Biosci. 13:1003-17.

Woodman CB1, Collins SI, Young LS. 2007. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 7(1):11-22.

Xi, L.F., Kiviat, N.B., Hildesheim, A., Galloway, D.A., Wheeler, C.M., Ho, J., Koutsky, L.A., 2006. Human papillomavirus type 16 and 18 variants: race-related distribution and persistence. J Natl Cancer Inst. 98 (15), 1045-1052. doi: 10.1093/jnci/djj297.

Xi, L.F., Koutsky, L.A., Hildesheim, A., Galloway, D.A., Wheeler, C.M., Winer, R.L., Ho, J., Kiviat, N,B., 2007. Risk for high-grade cervical intraepithelial neoplasia associated with variants of human papillomavirus types 16 and 18. Cancer Epidemiol Biomarkers Prev. 16 (1), 4-10. doi: 10.1158/1055-9965.EPI-06-0670.

Yamada T, Manos MM, Peto J, Greer CE, Munoz N, Bosch FX, Wheeler CM. 1997. Human papillomavirus type 16 sequence variation in cervical cancers: a worldwide perspective. J Virol. ;71(3):2463-72.

Yamada K, Ono M, Bensaddek D, Lamond AI, Rocha S, 2013a. FMN2 is a novel regulator of the cyclin-dependent kinase inhibitor p21. Cell Cycle. 12(15):2348-54. doi: 10.4161/cc.25511. Epub 2013 Jul 1.

Yamada K, Ono M, Perkins ND, Rocha S, Lamond AI. 2013b. Identification and functional characterization of FMN2, a regulator of the cyclin-dependent kinase inhibitor p21. Mol Cell. 49(5):922-33. doi: 10.1016/j.molcel.2012.12.023. Epub 2013 Jan 31.

Zanier K, ould M'hamed ould Sidi A, Boulade-Ladame C, Rybin V, Chappelle A, Atkinson A, Kieffer B, Travé G. 2012. Solution structure analysis of the HPV16 E6 oncoprotein reveals a self-association mechanism required for E6-mediated degradation of p53. Structure. 20(4):604-17. doi: 10.1016/j.str.2012.02.001. Epub 2012 Apr 3.

Zehbe, I., Lichtig, H., Westerback, A., Lambert, P.F., Tommasino. M., Sherman, L., 2011. Rare human papillomavirus 16 E6 variants reveal significant oncogenic potential. Mol Cancer. 10, 77. doi: 10.1186/1476-4598-10-77.

Zehbe, I., Richard, C., DeCarlo, C.A., Shai, A., Lambert, P.F., Lichtig. H., Tommasino, M., Sherman, L., 2009. Human papillomavirus 16 E6 variants

differ in their dysregulation of human keratinocyte differentiation and apoptosis. Virology. 383 (1), 69-77. doi: 10.1016/j.virol.2008.09.036.

Zimmermann H, Degenkolbe R, Bernard HU, O'Connor MJ, 1999. The human papillomavirus type 16 E6 oncoprotein can down-regulate p53 activity by targeting the transcriptional coactivator CBP/p300. J Virol. 73(8):6209-19.

zur Hausen H. 2002Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2(5):342-50

Anexo. Artículo publicado

Changes in global gene expression profiles induced by HPV16 E6 oncoprotein variants in cervical carcinoma C33-A cells