DETECTION OF HIGH-RISK HPV-E6 ONCOPROTEIN IN LOW AND HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN): EMERGING EVIDENCE FOR PREDICTIVE VALIDITY

Johannes Schweizer,[1] Chamorro Somoza,[1] Charles Mahoney,[1] Jon Silver,[1] Roger Peck,[2] Bernhard Weigl,[2] You-lin Qiao,[3] John Sellors,[2] and Peter S. Lu[1]

[1]Arbor Vita Corporation, Sunnyvale, CA, USA [2] PATH, Seattle, WA, USA [3] Cancer Institute, Chinese Academy of Medical Sciences, Beijing, PRC

INTRODUCTION

High-risk HPV types cause over 99% of cervical cancers. Expression of high-risk HPV oncoproteins E6 and E7 is necessary for cervical epithelial cell transformation, cancer progression and maintenance. Therefore, E6 oncoprotein is potentially a diagnostic marker with predictive validity (figure 4, schematic).

E6 of **all** high-risk HPV types contain a PDZ ligand motif (PL) and bind PDZ domains, but low risk HPV-E6 do **not** bind PDZs (figure 1). PDZs are conserved protein domains with widespread biological functions that include cell-to-cell contact, intercellular signaling, and cell polarity.

Specific binding of all high-risk HPV-E6 to PDZ is the basis of a novel, cervical cancer diagnostic rapid immunoassay that Arbor Vita Corporation develops in collaboration with PATH. Proof of concept studies demonstrate detection of HPV16-E6 from less than 31,000 cervical cancer cells (figure 2). The assay promises to be highly predictive of malignant transformation because it measures E6 oncoprotein, a primary cause of transformation.

RESULTS

Correlation of E6 expression with severity of cervical lesions.

Cervical swab specimen collected from women screened, diagnosed, and managed in rural settings in China were tested for the presence of HPV-16 DNA then examined for HPV16-E6 expression. E6 expression was found in 6 out of 7 CIN3 specimens, in 1 out of 2 CIN2 specimens, and in 1 out of 9 CIN1 specimens. Clients diagnosed with histologically confirmed CIN1 at baseline were enrolled in a cohort study where they were examined at one and two years. Year one follow-up included liquid-based cytology, HPV DNA testing via Hybrid Capture 2, and reflex colposcopy, biopsy, and ECC. Year two follow-up included liquid-based cytology, HPV DNA testing via Hybrid Capture 2, colposcopy, random and directed biopsy, and ECC. Most interestingly, the client with the E6 expressing CIN1 specimen progressed to CIN3 upon a two year follow up examination via random biopsy. No progression to CIN3 was seen for baseline CIN1 clients whose specimens did not express E6 (figure 3 and table in figure 4). Comparison to E6 levels in SiHa cervical cancer cells allows rough quantitation of E6 in cervical swab specimen.

Correlation of E6 expression with E6 DNA copy number

E6 DNA copy number varies widely among different cervical cancer cell lines, as reported previously. E6 expression in cervical cancer cell lines was tested by Western technology (figure 4, bottom). We find that E6 levels do not correlate with E6 DNA copy number (compare SiHa and CasKi). Rather, E6 oncoprotein appears to be expressed to very similar extent in different cervical cancer cell lines. Rough quantitation revealed the cervical cancer cell lines to contain roughly 1 ng / E6 per $1x10^6$ cells.

Development of a lateral flow based cervical neoplasia assay.

A lateral flow cervical cancer diagnostic assay based on the above described principle (figures 1, 2, and 5) is in development. At the current stage, E6 from ~ 150,000 SiHa cervical cancer cells can be detected visually in the background of HPV negative cervical swab material. Based on E6 quantities found in CIN3 clinical specimen and cervical cancer cell lines (figure 3), an assay target sensitivity of ~ 50,000 cells was determined.

CONCLUSIONS

• E6 is a potential diagnostic marker with predictive validity

Albeit sample numbers are small, first studies performed on cervical swab specimen collected from women in rural areas in China indicate a correlation between E6 expression and severity of disease (CIN1, 2 or 3) as well as the risk to develop severe lesions. These preliminary data are consistent with our current understanding of the natural history of cervical neoplasia and support the hypothesis that E6 is a diagnostic marker with predictive validity.

• E6 based diagnostic test for cervical pre-cancer and cancer

Arbor Vita Corporation and PATH have developed a prototype of an E6 oncoprotein based novel rapid diagnostic assay for cervical pre-cancer and cancer. All high-risk HPV-E6 are specifically captured by a PDZ domain protein; detection occurs via anti E6 mAb. The assay format is lateral flow ("strip test"). Current sensitivity is ~ 150,000 SiHa cells.

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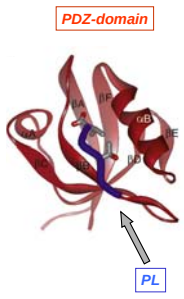
Figure 1

Only High-Risk HPV-E6 Bind PDZ Domains

HPV strain	E6 C-terminal sequence	PL YES / NO	Oncogenic
HPV 4	GYCRNCIRKQ	No	No
HPV 11	WTTCMEDLLP	No	No
HPV 20	GICRLCKHFQ	No	No
HPV 24	KGLCRQCKQI	No	No
HPV 28	WLRCTVRIPQ	No	No
HPV 36	RQCKHFYNDW	No	No
HPV 48	CRNCISHEGR	No	No
HPV 50	CCRNCYEHEG	No	No
HPV 16	SSRTRRETQL	Yes	Yes
HPV 18	RLQRRRETQV	Yes	Yes
HPV 31	WRRPRTETQV	Yes	Yes
HPV 35	WKPTRRETEV	Yes	Yes
HPV 30	RRTLRRETQV	Yes	Yes
HPV 39	RRLTRRETQV	Yes	Yes
HPV 45	RLRRRRETQV	Yes	Yes
HPV 51	RLQRRNETQV	Yes	Yes
HPV 52	RLQRRRVTQV	Yes	Yes
HPV 56	TSREPRESTV	Yes	Yes
HPV 59	QRQARSETLV	Yes	Yes
HPV 58	RLQRRRQTQV	Yes	Yes
HPV 33	RLQRRRETAL	Yes	Yes
HPV 66	TSRQATESTV	Yes	Yes
HPV 68	RRRTRQETQV	Yes	Yes
HPV 69	RRREATETQV	Yes	Yes
HPV 26	RPRRQTETQV	Yes	Yes
HPV 53	RHTTATESAV	Yes	Yes
HPV 73	RCWRPSATVV	Yes	Yes
HPV 82	PPRQRSETQV	Yes	Yes



PL motif: X-S/T-X-V/L

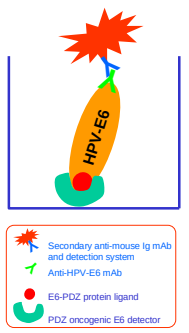
100% Structural Specificity

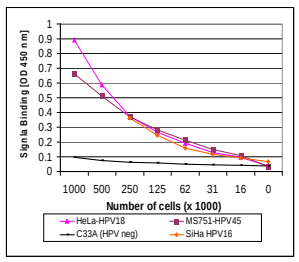
- E6 oncoprotein of **all** known high-risk HPV types has a C-terminal PDZ binding motif (PL) for PDZ ligand; none of the low-risk HPV have a PL. This correlation is 100% complete (left). Ribbon rendering of a PL bound to a PDZ domain (right).

(Picture from: HARRIS & LIM. (2001) J CELL SCI 114:3219-31)

Figure 2

PDZ Based High-Risk HPV-E6 Detection

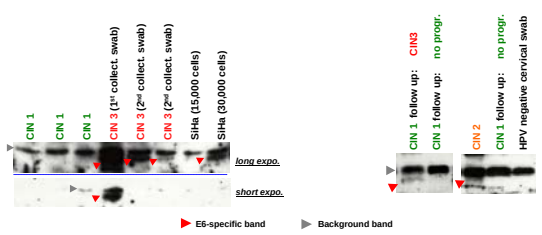




- Arbor Vita's E6 based cervical cancer diagnostics: specific capture of high-risk HPV-E6 via a PDZ domain and detection via anti E6 mAb (left).
- Applying the sandwich ELISA principle shown left, E6 is detected in cervical cancer cell lines. Limit of E6 detection is < 31000 cells (right).

Figure 3

E6 Levels Correlate with Severity of Lesions



- Cervical swab specimen of women diagnosed with CIN1 or CIN3 were tested for presence of HPV16-E6 oncoprotein via PDZ based E6 "pull down" (method below). 2 of 3 CIN3 swabs were "secondary" collection swabs.
- Patients with CIN1 pathology are followed up for further disease progression; first results suggest that E6 oncoprotein expression in CIN1 correlates with high-risk for progression to a more severe state.

PDZ domain protein based "pull down" of high-risk HPV-E6 by a PDZ

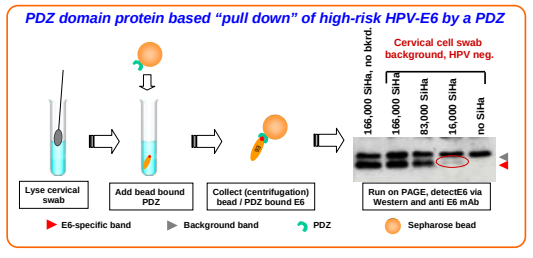
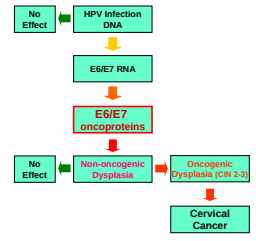


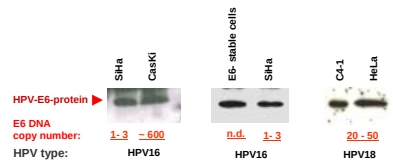
Figure 4

E6 - Marker of High Positive Predictive Value



Pathology	Specimen tested	E6 positive	Year 2 follow-up
CIN1	9	1	E6 pos.: CIN1 to CIN3; E6 neg.: no prog.
CIN2	2	1	N.D.
CIN3	7	6	N.D.
CxCa (tumor)	2	2	N.D.

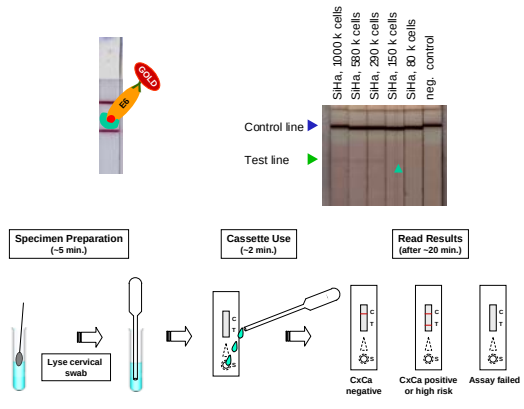
Selected experimental data shown in figure 3



- Cervical epithelial cell transformation depends on expression of E6 and E7 oncoproteins. E6 levels correlate with severity of CIN lesions (table).
- Expression of E6 oncoprotein in different cervical cancer cell lines (~ 1 ng / 10^6 cells), is not correlated with highly variable HPV-E6 DNA copy numbers (bottom).

Figure 5

E6 Based Lateral Flow CxCa Diagnostics



- Specific capture of high-risk HPV-E6 via a PDZ domain and detection via anti E6 mAb applied to lateral flow assay format (top left).
- Detection of HPV16-E6 from cervical cancer cells (SiHa) lines in the background of a HPV negative cervical swab specimen (top right).
- Workflow illustration of a cervical cancer rapid diagnostic diagnostic assay (bottom).

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