DEVELOPMENT OF A DIAGNOSTIC ASSAY BASED ON THE BINDING OF HIGH-RISK HPV-E6 ONCOPROTEINS TO PDZ

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BACKGROUND

HPV-E6 is a viral oncoprotein involved in protein-protein interaction. The name "PDZ" relates to the first three proteins discovered to contain PDZ domains: PKD, CASK, and Dlg1. More than 200 different PDZ domains have been identified in eukaryotes, conserved cellular functions that include cell-to-cell contact, intercellular signaling, and cell polarity. PDZ domains extend over approximately 100 residues, but the basic PDZ binding motif, referred to as "YXX" or "FXQ," consists of the four most conserved amino acids on proteins that bind to PDZ. An example for a classical YXX motif is YXXQ. Numerous viral protein domains have PXX motifs that can bind to PDZs. For example, all high-risk HPV-E6 proteins can bind PDZs.

INTRODUCTION

High-risk HPV types cause over 99% of cervical cancers. Accumulating evidence suggests that recognition of high-risk HPV-E6 proteins depends on HPV-E6 protein binding to cellular PDZ domains. PDZ domains contain a highly conserved sequence motif: X-(V/1)-X-O, where the "X" represents any amino acid and the "O" represents a conserved proline. This PDZ motif is critical for high-risk HPV-E6 protein interaction with cellular PDZ domains. PDZ domains are conserved protein domains involved in protein-protein interaction. The name "PDZ" relates to the first three proteins discovered to contain PDZ domains: PKD, CASK, and Dlg1. More than 200 different PDZ domains have been identified in eukaryotes, conserved cellular functions that include cell-to-cell contact, intercellular signaling, and cell polarity. PDZ domains extend over approximately 100 residues, but the basic PDZ binding motif, referred to as "YXX" or "FXQ," consists of the four most conserved amino acids on proteins that bind to PDZ. An example for a classical YXX motif is YXXQ. Numerous viral protein domains have PXX motifs that can bind to PDZs. For example, all high-risk HPV-E6 proteins can bind PDZs.

The specific aim of this study was to determine whether high-risk HPV-E6 proteins can bind PDZ domains. This was accomplished by developing a novel PDZ-based cervical cancer diagnostic assay. The assay is designed to detect high-risk HPV-E6 proteins that bind to cellular PDZ domains. The assay is based on the principle that high-risk HPV-E6 proteins bind to cellular PDZ domains, whereas low-risk HPV-E6 proteins do not. The assay is simple, rapid, portable, and of moderate throughput.

RESULTS

Components of a novel cervical neoplasia diagnostic assay: "PDZ oncoprotein E6 detector" and anti-mouse monoclonal antibody (anti-mAb).

The aim of this study was to develop a cervical cancer diagnostic assay that is simple, rapid, portable, and of moderate throughput. A prototype of a novel cervical cancer diagnostic assay was developed that is based on the specific recognition of all high-risk HPV-E6 via a PDZ domain, the "PDZ oncoprotein E6 detector" (PVCA-326) and detection via mAb. The assay is simple, rapid, portable, and of moderate throughput. The assay was developed by Arbor Vita Corporation and PATH.

Binding of recombinant HPV-E6 proteins representing five high-risk HPV types (16, 18, 35, 45, and 56) was determined by Western. For example, recombinant HPV-E6 only detect PDZ domains that include cell-to-cell contact, intercellular signaling, and cell polarity. PDZ domains extend over approximately 100 residues, but the basic PDZ binding motif, referred to as "YXX" or "FXQ," consists of the four most conserved amino acids on proteins that bind to PDZ. An example for a classical YXX motif is YXXQ. Numerous viral protein domains have PXX motifs that can bind to PDZs. For example, all high-risk HPV-E6 proteins can bind PDZs.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

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A) E6 oncoprotein of all known-risk HPV has a C-terminal PDZ binding motif (E6 PDZ). B) PDZ ligand of the high-risk HPV-E6 has an amino acid sequence (AVC PDZ88) that binds E6 with the highest affinity (Fig. 2). Specific binding of all high-risk HPV-E6 to PDZ capture of high-risk HPV-E6 only.

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