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The Structural and Dynamic Response of MAGI-1 PDZ1 with Noncanonical Domain Boundaries to the Binding of Human Papillomavirus E6

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PDZ domains are protein interaction domains that are found in cytoplasmic proteins involved in signaling pathways and subcellular transport. Their roles in the control of cell growth, cell polarity, and cell adhesion in response to cell contact render this family of proteins targets during the development of cancer. Targeting of these network hubs by the oncoprotein E6 of "high-risk" human papillomaviruses (HPVs) serves to effect the efficient disruption of cellular processes. Using NMR, we have solved the three-dimensional solution structure of an extended construct of the second PDZ domain of MAGI-1 (MAGI-1 PDZ1) alone and bound to a peptide derived from the C-terminus of HPV16 E6, and we have characterized the changes in backbone dynamics and hydrogen bonding that occur upon binding. The binding event induces quenching of high-frequency motions in the C-terminal tail of the PDZ domain, which contacts the peptide upstream of the canonical X-[T/S]-X-[L/V] binding motif. Mutations designed in the C-terminal flanking region of the PDZ domain resulted in a significant decrease in binding affinity for E6 peptides. This detailed analysis supports the notion of a global response of the PDZ domain to the binding event, with effects propagated to distal sites, and reveals unexpected roles for the sequences flanking the canonical PDZ domain boundaries.

Introduction

PDZ domains are protein–protein interaction domains that are commonly found in cytoplasmic proteins involved in signaling pathways or subcellular transport.[1–3] PDZ domains play roles in localizing proteins to the membrane and in acting as molecular scaffoldings or adaptors, but also serve in other functions such as binding to titin Z-repeats

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Abbreviations used: HPV, human papillomavirus; NOE, nuclear Overhauser enhancement; BMRB, BioMagResBank; PDB, Protein Data Bank; SPR, surface plasmon resonance; TOCSY, total correlated spectroscopy; NOESY, NOE spectroscopy; GST, glutathione *S*-transferase; RU, response units; RDC, residual dipolar coupling.

in the Z-disk of sarcomeres[4] or detecting unfolded proteins and activating proteases.[5] Many PDZ-domain-containing proteins are located at the interface between the cytoskeleton and the cellular membrane, where they are implicated in the formation of cellular junctions such as synapses,[6] adherens junctions, or tight junctions.[7] They commonly form multiprotein complexes at the inner interface of the membrane and are associated with the control of cell growth, cell polarity, and cell adhesion in response to cell contact and thus represent a family of proteins targeted during the development of cancer.[8] Multiple PDZ domains are often found within a single protein connected by linkers of varying lengths, and found to be associated with other domains such as WW and SH3 domains, reflecting multiple functions. This further suggests that the composite protein function may be more than the sum of individual domains.

Viral proteins often target PDZ domains, resulting in the disruption of cellular processes for the benefit of the viral life cycle. The targeting of PDZ-domain-containing proteins involved in cellular adhesion and control of polarity has been shown to be a highly important activity in the process of cancer development following infection by "high-risk" human papillomaviruses (HPVs).[9–11] The expression of two HPV oncoproteins, E6 and E7, that cooperate in cell immortalization and transformation has been associated with tumorigenesis.[12,13] E6 has a number of distinct functions in the host cell. It targets the tumor suppressor p53 for degradation through the formation of a trimeric complex with the cellular ubiquitin ligase E6AP[14] and represses p53-dependent cell-cycle control and p53-dependent transcription by inhibiting p300-mediated acetylation.[15] In addition, E6 binds and sometimes drives the proteasome-mediated degradation (*via* PDZ domain recognition) of several PDZ-domain-containing proteins. These include various MAGUKs (*m*embrane-*a*ssociated *gu*anylate *k*inases), such as Dlg-1[16], Dlg-4,[17] and hScrib,[18] and MAGI (*m*embrane-*a*ssociated *g*uanylate kinase with *i*nverted domains) proteins, such as MAGI-1[19], MAGI-2, and MAGI-3[20] (Fig. 1a). The tumorigenic effects of HPV E6 depend partly on interfering with MAGI-1 functions in the living cell.[19] Several non-MAGUK proteins such as CAL,[21] MUPP-1[22], PATJ,[23] PTPN3,[24] Tip1,[25] and Tip2[26] are also targeted by E6. The interaction of high-risk HPV E6 proteins with PDZ domains is mediated by C-terminal peptide sequences matching the X-[T/S]-X-[L/V] motif of "class I" PDZ domains[27–29] and is associated with the development of cervical cancer.[29,30] This consensus sequence is only found in high-risk HPV E6s (such as HPV16 or HPV18) that differ in their C-terminal residues and, as a result, exhibit different affinities for MAGI-1.[8,31,32] A higher affinity seems to correlate with an increased likelihood of recurrence and metastasis in cervical tumors.[20,33] This variability in the C-terminal residue of HPV E6 proteins has been suggested to be important in fine-tuning the affinities of the viral oncoproteins for distinct sets of PDZ domains.[9]

Understanding how the E6 viral protein interferes with the endogenous network of interactions mediated by PDZ domains requires both knowing the general rules governing PDZ–peptide interactions and deciphering the specific strategies used by the virus during infection. In the past few years, many studies attempted to characterize the ligand binding "specificity code" of PDZ domains. Initial analysis of peptide library screens, combined with sequence analysis to identify C-terminal consensus binding sequences, led to the definition of three major PDZ domain classes.[2,34] Later, the mapping of 3100 peptides identified by phage display against 82 PDZ domains from worms and humans allowed a more precise analysis of binding specificity and resulted in a classification of PDZ domains into 16 distinct specificity classes.[35] A study of the binding selectivity of 157 PDZ domains from the mouse proteome using protein microarrays and quantitative fluorescence polarization[36] showed that selectivity is derived from interactions throughout the binding pocket. This led the authors to suggest that, in terms of binding selectivity, PDZ domains constitute a continuum rather than discrete classes.

At the molecular level, a large number of three-dimensional structures of PDZ domains have been determined,[37] with a number of them being available in both unliganded and liganded forms, allowing structural changes induced by peptide binding to be studied. In most cases, the structure of PDZ domains displayed a very small change upon peptide binding.[38,39] However, from a dynamic point of view, computational[40–45] approaches have highlighted the role of regions distal to the peptide binding site in forming dynamic networks within PDZ domains, and a number of studies have characterized changes in dynamics using NMR, thus providing experimental evidence for these networks.[39,46–48]

Initial structural insight into the targeting of PDZ domains by HPV E6 protein was provided by the crystal structures of a short peptide from HPV18 E6 bound to three PDZ domains from MAGI-1 and SAP97/Dlg.[49] This work revealed that peptide residues outside the canonical PDZ binding motif were involved in direct contacts with the canonical core regions of the PDZ domains. In addition, constructs of MAGI-1 PDZ1 [the second of the six PDZ domains in the sequence of human MAGI-1; residues 456–580 of human MAGI-1 (GenBank accession no. AF401656) when referring to the specific polypeptide used in these studies] were seen to form covalent cysteine-bridged dimers both in solution and in the crystal.