Identification of Protein Partners of the APC Tumor Suppressor.

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Introduction:
Mutations in the adenomatous polyposis coli (APC) gene are responsible for a form of colon cancer known as familial adenomatous polyposis coli (FAP). These individuals develop hundreds to thousands of polyps in the large intestine at an early age. If these tumors are not surgically removed they invariably progress to colorectal cancer. Most of the knowledge about APC function has been gained from studies examining its protein partners. The APC gene product contains binding sites for beta-catenin, the human discs large protein, microtubules, and a protein of unknown function, EB1.

Using the yeast two-hybrid system, two putative proteins partners of APC were identified by screening a cDNA library made of the human brain. This library was chosen since mutations in APC are known to result in CNS tumors and because several of the alternative transcripts of APC appear to be highly expressed in neuronal tissues. The proteins identified were KIAA 0454 and pM5.

The major problem with the yeast two-hybrid assay is the tendency to obtain false-positive results. As such, all positive results must be confirmed by demonstration of the interaction using independent biochemical means.

Hypothesis:
The APC protein binds to KIAA 0454 and/or pM5.

Methods:
In order to determine whether the APC protein and pM5 interact in vivo, the cDNA's for APC (full-length and BS-APC) and pM5 were cloned into eukaryotic expression vectors and then cotransfected into COS-1 cells. Immunoprecipitation and Western analyses were then performed to assay for in vivo interactions.

To determine whether the APC protein and KIAA 0454 interact in vivo, the cDNAs for -APC (full-length and BS-APC) and KIAA 0454 were cloned into eukaryotic expression vectors and then cotransfected into COS-1 cells. Immunoprecipitation and Western analyses were then performed to assay for in vivo interactions.

Results:
APC full-length, BS-8 APC, and BS-14 APC have each individually been cloned into the expression vector pEGFP. pM5 has been successfully cloned into the expression vector pcDNA 3.1. Attempts in cloning KIAA 0454 into the expression vector have been unsuccessful thus far. Each of the APC constructs and the pM5 construct have been cotransfected into COS-1cells. Progress is currently being made in the immunprecipitation and western analysis of the protein lysate collected from these cotransfected cells.

Conclusions:
If either KIAA 0454 or pM5 is a true protein partner of APC, study of this interaction would

yield important new information about how APC acts as a tumor suppressor and perhaps shed light on any unique role for the BS isoform of APC.