Modulating Expression of the APC Tumor Suppressor Using RNA Interference

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APC is a tumor suppressor gene that is mutated in the germline of individuals with Familial Adenomatous Polyposis Coli (FAP), a disease in which numerous benign polyps carpet the colon and without a colectomy, development of colon cancer is inevitable. In addition to inherited colon cancer, chain-terminating mutations in *APC* are found in approximately 50% of sporadic colorectal adenomas and 80% of adenocarcinomas. Mutations in *APC* also have been detected in other tumor types, including up to 25% of sporadic breast cancers. Although much is known about the consequence of re-introducing APC into colorectal cancer cells and mutating Apc in genetically engineered mouse models, very little is known about its molecular function in normal epithelial cells. In this work, we sought to test the hypothesis that by reducing the levels of APC in nontransformed epithelial cells, we will be able to determine its normal function as a tumor suppressor and the consequence of *APC* mutation on several cellular processes including proliferation, survival and cell-cell interactions. Our preliminary data using antisense technology to reduce APC levels demonstrated that APC plays a role in cell-cell contact and polarization of epithelial cells. Here, we report the development an adenoviral-based RNA interference method using siRNAs (small interfering RNAs) to down-regulate APC. In this approach, a small nucleotide sequence of the gene of interest is introduced into cells via an adenoviral delivery vector; it binds to mRNA transcripts and degrades them, effectively reducing protein levels in cells. Advantages of using such a delivery system include that it produces a highly efficient but transient down-regulation of the gene product of interest. In these experiments, an *APC* oligonucleotide (nucleotides 114-144) was subcloned into the siRNA expression vector pSiren-shuttle. This plasmid, *pSiren-APC*, was transiently transfected into COS-1 African green monkey kidney cells for 24 to 48 hours. Total RNA was harvested from transfected cells, and APC expression was analyzed using reverse-transcriptase PCR. Preliminary results suggested that there was some reduction of APC in COS-1 cells transfected with *pSiren-APC*, although it is likely that the transfection conditions were not optimized for this cell line. Currently, this construct is being subcloned into adenoviral DNA for production of virus expressing the APC siRNA. These results suggest that RNA intereference is a promising strategy to modulate APC expression in epithelial cells in order to elucidate its function as a tumor suppressor *in vitro* and, possibly, *in vivo*.