β-Catenin Mutations: Insights into the APC Pathway and the Power of Genetics

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See related article by Sparks et al., Cancer Res 1998;58:1130–4.

Cancer Genetics and *APC* in the Pre-Genome Era

In the mid 1990s, the human genome project was well underway, but it would still be more than 5 years before the first draft of the human genome was published and more than 20 years before the first cancer genomes were deciphered. Nevertheless, genetic studies of cancer had already yielded significant insights, with many of the major cancer driver genes (e.g., *TP53*, *KRAS*, *PTEN*, and *RB*) we recognize today having already been discovered. One of these major cancer genes was the *APC* tumor suppressor gene, whose existence was first suggested by the study of familial adenomatous polyposis (FAP). FAP patients develop hundreds to thousands of colorectal adenomas, the benign precursors to colorectal cancer. Paradoxically, one of the first clues to the location of the gene responsible for FAP came from the study of a patient with noninherited adenomatous polyposis who had cytogenetically visible interstitial deletion of 5q (1). This observation was quickly confirmed and extended by linkage analysis, which localized the *FAP* gene to chromosome 5q21 (2, 3). Although FAP patients account for less than 1% of all colorectal cancers, a series of classic studies using loss of heterozygosity (LOH) suggested that a gene on 5q might also be involved in the development of sporadic colorectal cancer (4, 5). These two lines of evidence converged and bore fruit in 1991, when Ray White and colleagues and our group in collaboration with Yusuke Nakamura cloned the *APC* tumor suppressor gene (6–8). These initial reports and follow-up studies firmly established *APC* gene mutations as being responsible for the vast majority of FAP and sporadic colorectal cancers, with the former being due to inherited mutations and the latter to somatic mutations. With the completion of the human genome and advancements in sequencing technology, it is now possible to recapitulate these studies in a few days or less. However, the fact that many of the major cancer driver genes were already identified in the pre-genome era is a testament to the efforts and dedication of research groups around the world.

β-Catenin and the APC Pathway

One of the major challenges then and now is understanding how these cancer driver pathways function in normal cells and how this function is corrupted in cancer. This was certainly the case for the *APC* gene, which encodes a 2843 amino acid protein with multiple domains. Early studies had already identified numerous interactions at the protein level [e.g., catenins (9–11), microtubule (12, 13), EB1 (14), GSK3β (15), hDLG (16)], but whether any of these interactions played a role in APC tumor-suppressive function was unclear. As our title foretells, the catenin interactions were of particular interest. Catenins were originally identified as proteins that bind the cytoplasmic tails of cadherin proteins, a family of transmembrane proteins involved in homotypic cell–cell contacts. One of the catenin members, β-catenin, was particularly interesting, as it was found to be homologous to *Armadillo*, a segment polarity gene in *Drosophila* that is crucial in developmental signaling. In *Drosophila*, *Armadillo* interacts with the transcription factor Pangolin (or *Drosophila* TCF) to activate transcription of downstream targets that control development. However, the role of β-catenin in signaling in higher eukaryotes remained unclear until it was shown that β-catenin was involved in the signaling that specifies dorsal–ventral development in *Xenopus laevis* (*X. Laevis*; reviewed in ref. 17). In a functional study that presaged findings in human cancer, Yost and colleagues showed that β-catenin phosphorylation in its amino terminus led to its degradation and reduced signaling (18). Mutating these sites to nonphosphorylatable residues increased β-catenin levels and resulted in constitutive activation of the pathway. β-Catenin was therefore shown to be a crucial player of Wnt signaling.

In this context, the interaction between APC and β-catenin provided an intriguing link between these two important pathways. Might APC be involved in Wnt signaling? Might β-catenin be important in colorectal cancer development? Several groups, including our own, endeavored to answer these important questions. Paul Polakis and colleagues reported that β-catenin levels were downregulated through its interaction with APC (19). By analogy with the findings in *X. laevis*, this suggested that an important function of APC might be to downregulate β-catenin–mediated signaling. This also suggested that an alternative approach to the activation of the APC pathway in colorectal development might be β-catenin stabilization through mutation of its N-terminal phosphorylation sites. Using TCF reporter plasmids that could measure β-catenin–mediated transcriptional activity, Hans Clevers' laboratory in collaboration with our group demonstrated that APC could indeed downregulate β-catenin signaling (20, 21). Strikingly, mutations in the β-catenin phosphorylation sites that render it resistant to APC inhibition were identified in colorectal cancer and melanoma (21, 22). While these findings provided strong evidence that an important function of APC was to downregulate the WNT pathway through its ability to bind β-catenin and decrease its levels, they did not constitute an absolute proof. We believed that a genetic approach might provide further evidence that APC is upstream of β-catenin in WNT signaling. We hypothesized that if these two genes are in the same pathway, mutations of these genes should be mutually

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exclusive. In other words, once the pathway is activated by a mutation in one of these genes, there would be no selective pressure for tumors to select for a mutation in the other gene. In our previous work, we had tantalizing hints that these mutations may indeed be mutually exclusive, but we believed that an in-depth genetic analysis might provide formal proof, which led to the 1998 study (23) published in *Cancer Research*.

In that article, we showed that although β-catenin gene (*CTNNB1*) mutations were frequent (48%) in colorectal cancers lacking an *APC* mutation, they were extremely rare in tumors with mutant *APC*. In fact, none of the 28 tumors with a known *APC* mutation was found to contain a *CTNNB1* mutation in exon 3, the exon that encodes the phosphorylation sites. This mutually exclusive distribution was found to be highly statistically significant ($P < 3 \times 10^{-4}$). Our study provided near incontrovertible evidence that APC and β-catenin were part of the same oncogenic pathway in colorectal cancer and that mutation of only one of these proteins (activation of β-catenin or inactivation of *APC*) was sufficient to fully activate the pathway. In addition, *CTNNB1* mutations were found in early adenomas in a mutually exclusive manner with *APC* mutations, consistent with the previously established early role for the APC pathway in colorectal cancer development. This analysis exemplifies the power of genetics in establishing functional relationships in human cancer, as it would be near impossible to reach this level of certainty through biochemical or functional studies. Although biochemical and functional studies are very important at illuminating "possible" routes to neoplastic conversion, studying the mutations selected by human tumors allows us to define the "actual" routes.

Genetic Insights into Pathways and Biochemical Function

Our article was one of the early studies illustrating that mutation patterns can not only implicate cancer driver genes but can also provide important insights into pathways and key functions. Other pairs of "mutually exclusive" mutations have since been reported in various cancers. For example, BRAF and KRAS were found to be mutually exclusive in colorectal cancer (24), and BRAF and NRAS in melanoma (25). With the advent of large-scale cancer genome sequencing, this approach has been frequently used to systematically define cancer driver pathways though analysis of large cancer mutation datasets (evaluated in ref. 26). By studying the patterns of exclusivity, these methods can identify, with high degree of probability, not only two, but multiple genes that are part of a cancer pathway. Moreover, these statistical approaches, based on "mutual exclusivity," allow the identification of genes that would not be found using frequency-based methods.

In the post-genome era, where more than 4 million mutations have been identified in over 25,000 sequenced cancer genomes, it is clear that the pre-genome era hypotheses of a mutated gene equals a cancer driver gene does not hold. This is largely because the mutations are a result of random processes, resulting in a background mutation rate targeting every gene in the genome. Defining drivers by accounting for this background rate is one solution, but variation with repair processes across the genome, tissue types, and environmental exposures make this challenging. Biochemical and functional studies can be helpful, but they can only define the realm of possibilities, and can even be misleading. Fortunately, as explained above, patterns of somatic mutations can provide the answer. Background and driver mutations are the result of random processes, but biological selection of driver gene mutation produces recognizable patterns of mutations that can be used to define drivers and pathways. The β-catenin/APC connection presented in our *Cancer Research* article was one of the earliest relationships identified through this approach.

Disclosure of Potential Conflicts of Interest

K.W. Kinzler has ownership interest (including patents) in PGDx and PapGene and is a consultant/advisory board member for Sysmex Inostics. No potential conflicts of interest were disclosed by the other authors.

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