Cancer Biology

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Published in:
Current Biology

DOI:
10.1016/j.cub.2017.07.028

Publication date:
2017

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Document Version
Peer reviewed version

Title:
Cancer Cell Biology: The Adenomatous polyposis coli tumor suppressor delivers the kiss of death to focal adhesions.

Summary:
The actin nucleating ability of the Adenomatous polyposis protein is required for disassembling focal adhesions. Loss of this function in *Apc* mutant cells reduces directed cell migration. This can explain the reduced migration of colon cancer cells.

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Usually, tumor suppressors are associated with signaling pathways that directly impact on transcription and gene expression profiles to increase proliferation and survival. The major tumor suppressor in colon cancer is unusual in this regard. It has many different functions and is increasingly recognized as an important cytoskeletal regulator. A new study by Bruce Goode's team at Brandeis University provides new mechanistic insights into this role and its importance for epithelial cell behavior, particularly directed cell migration and the associated turnover of focal adhesions [1].

The adenomatous polyposis coli (*Apc*) gene is mutated in most tumors of the lining of the intestinal tract and these mutations occur at the earliest stages of cancer initiation [2, 3]. The APC protein has received much attention in the context of regulating the activity of Wnt signaling, which dominates decisions about proliferation in the colonic epithelium [4]. Wnt is particularly important for stem cells and their survival [4]. However, unlike other tumor suppressors, APC is also physically linked to many other cellular machineries. Most notably it has direct interactions with all cytoskeletal proteins and some of their regulators [5]. The large molecular size of APC (2,843 amino acids in human) and the complexity of its interactions have made it difficult to separate its contributions to Wnt

signaling and cytoskeletal regulation [5]. Nonetheless, the consequences of APC cytoskeletal interactions for cell behavior and how changes in these processes contribute to cancer when APC is mutated are understood best for its effect on microtubules [6, 7]. APC binds to and bundles microtubules and this contributes to normal mitotic spindles and cell migration [6, 8]. Importantly, the interaction of APC with microtubules is mutually exclusive with its binding to β-catenin [9, 10]. That means not only truncation mutations in *Apc* that abolish its binding to microtubules, but also stabilizing mutations in β-catenin inhibit its contribution to microtubules stability and function [9, 10]. APC also interacts and cooperates with other microtubule +TIP binding proteins to form a superstructure that promotes the polymerization of microtubules [11]. APC also binds to actin. Initial studies showed that the same domain that binds directly to microtubules is involved in the binding and bundling of F-actin [12]. In the new study, a functional consequence of the interaction of APC with actin has been revealed and the domain involved has been narrowly defined [1]. Specifically, Juanes et al. show that the ability of APC to nucleate actin assembly is important for the normal turnover of focal adhesions. This can explain the deleterious effect of APC mutations on directed cell migration, which lead to loss of this domain.

Generating a uniquely mutated APC protein, the authors generate a separation-of-function mutant APC protein that is compromised only in this single activity and maintains normal interaction with and effect on microtubules *in vitro* and *in vivo*. APC that contains two single point mutations at residues 2,539 and 2,541 can rescue microtubules organization in APC depleted cells, but not their directed migration. This is likely due to the failure of focal adhesions to disassemble suggested by their persistence and retention of markers for mature focal adhesions. Expression of this mutant APC protein even in the presence wild type APC has the same effect suggesting that its association with wild type

APC creates non-functional protein complexes. Together with the inability of a corresponding APC protein fragment carrying these mutations to dimerize, this led to the hypothesis that dimerization of APC is a key factor in promoting the actin nucleation involved in focal adhesion disassembly.

This is not the first time APC has been linked to the regulation of focal adhesions. Deletion of *Apc* specifically in the intestinal epithelium leads to upregulation of focal adhesion kinase (FAK) [13]. Consistently, prolonged persistence of focal adhesions in cells carrying APC defective in actin nucleation is accompanied by persistent FAK activation [1].

What emerges is the idea that focal adhesion disassembly requires the delivery of APC by microtubules, likely as part of a +TIP complex, to focal adhesions where it engages in promoting actin nucleation locally to initiate their disassembly (Figure). Questions that now arise include how this handoff is orchestrated. Specifically, what are molecular events that facilitate the local switch from being part of the microtubule +TIP complex to engaging in actin nucleation. And how are proteins that regulate focal adhesions, particularly FAK, involved. The specific domain of APC that was identified in the new study is flanked by two direct microtubule binding sites of APC and its activity requires dimerization [9, 11]. The ability of APC to stimulate microtubule polymerization does not require its dimerization and is enhanced by the presence of other +TIPs [11, 14]. This makes it attractive to speculate that engagement of both microtubule domains, binding to other +TIPs, dimerization (or multimerization) are involved in the change from microtubule polymerization to actin nucleation (Figure). The idea that a complex interplay between different protein interactions can direct the function of APC locally, is also supported by the relationship between microtubule and β-catenin binding by APC. Binding of APC to microtubules and β-catenin is mutually exclusive although it involves distinct regions of the

APC molecule [9, 10]. Furthermore, the region of the APC protein involved in interactions with β-catenin, microtubules, F-actin and +TIPs is predicted to be unstructured [15]. This may allow APC to assume different conformations when associating with different binding partners and provide the means to allow APC to be segregated into different functions.

Altered migration is a commonly associated with tumor cells. Invasion of tumor cells to form metastases distant from the original tumor site is what is most commonly responsible for lethality. That means the idea that mutations in *Apc* that decrease cell migration are involved in tumorigenesis seems counterintuitive. However, in the intestinal epithelium decreased migration is absolutely required to initiate cells that can form tumors at least initially. Migration of cells in the colonic epithelium is particularly important for normal tissue homeostasis. Normally, proliferating cells produced by stem cells at the base of tissue invaginations called crypts of Lieberkühn, continually migrate upwards towards the lumen of the colon where they are exfoliated [16]. An important consequence of this highly dynamic turnover is that the lifetime of cells in the epithelium is extremely short. An increase in the residency time in the epithelium is absolutely required for a cell to form tumors in the intestinal epithelium. Indeed, decreased migration is a well-recognized consequence of *Apc* mutations in cultured cells and in intestinal tissue [6, 17]. Furthermore, computational models for tissue dynamics of crypts have shown that simply increasing the proliferation rate of cells is not sufficient to explain the ability of *Apc* mutant cells to preferentially colonize crypts over wild type cells [18]. Only by introducing increased retention of APC mutant cells, by making them more adherent or less migratory, could these models produce data matching *in vivo* results. The new study provides molecular confirmation for these models [1]. Combined with the increase in genetic instability

resulting from *Apc* mutations, prolonged retention of *Apc* mutant cells lays the foundation for the highly penetrant phenotype of *Apc* mutations [19].

In summary, much like "Dementors" deliver the kiss of death to people in the famous Harry Potter stories, APC delivers a kiss of death to focal adhesions [20]. Understanding how other protein interactions of APC keep this activity in check and how exchange between them is regulated will explain how cells conjure up a corresponding "Patronus" to protect them. Given the complexity of APC interactions, it is likely that, just like the different shapes these protective spirits assume, a number of different mechanisms are involved.

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