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Antagonistic crosstalk between APC and HIF-1α

Inke Näthke[1] and Sonia Rocha[2,*]

[1]Division of Cell and Developmental Biology; [2]Wellcome Trust Centre for Gene Regulation and Expression; College of Life Sciences; University of Dundee; Dundee, Scotland UK

Most colorectal cancers have mutations in the tumor suppressor APC. The best-understood function of APC is its participation in a protein complex that regulates the availability of β-catenin. Solid tumors are characterized by the presence of hypoxia as well as inflammation, which leads to the upregulation of Hypoxia Inducible Factors like HIF-1α. We recently demonstrated a novel antagonistic link between APC and HIF-1α. We found that hypoxia results in reduced levels of APC mRNA and protein via a direct HIF-1α-dependent mechanism. Similarly, APC mediates the repression of HIF-1α. However, this requires wild-type APC, low levels of β-catenin and NFκB activity. These results reveal the downregulation of APC as a novel mechanism that contributes to the survival advantage induced by hypoxia and cytokines such as TNFα. Our data indicate that loss-of-function mutations in APC result in the engagement of the hypoxia response. Importantly, this suggests that other stimuli that induce HIF, such as inflammatory cytokines and oncogenes, alter APC function.

Key words: HIF-1, APC, hypoxia, NFκB

Abbreviations: APC, adenomatous polyposis coli; HIF, hypoxia inducible factor; FAP, familial adenomatous polyposis; HRE, hypoxia responsive element; PHD, prolyl-hydroxylase; NFκB, nuclear factor κB; GEF, guanine-nucleotide exchange factor

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*Correspondence to: Sonia Rocha;
Email: s.rocha@dundee.ac.uk

Adenomatous polyposis coli (APC) is a tumor suppressor mutated in most colorectal cancers.[1] *APC* is also mutated in the human syndrome Familial Adenomatous polyposis (FAP). FAP patients are heterozygous for APC. They develop hundreds of polyps in their gut,[2,3] and progression to malignancy involves the presence of inflammation and hypoxia.[4,5] The APC protein is involved in many of the fundamental processes that govern normal gut epithelium. It is best known for controlling the Wnt/β-catenin pathway, where it regulates β-catenin levels, thus regulating the transcriptional activity of TCF/LEF transcription factors.[6] APC also contributes to the regulation of cytoskeletal proteins.[7]

Hypoxia is a common feature of solid tumors and regulates tumor angiogenesis and growth.[4,8] Hypoxia leads to the induction of the transcription factor Hypoxia Inducible Factor (HIF),[9] a heterodimeric transcription factor composed of α and β subunits. While HIF-1β is constitutively expressed, HIF-α subunits are extremely labile at normal oxygen levels. Oxygen controls HIF-α levels through post-translational hydroxylation, catalyzed by a class of 2-oxoglutarate dioxygenases called prolyl-hydroxylases (PHDs). Hydroxylation of HIF-α signals for the ubiquitin recognition complex containing the von Hippel Lindau tumor suppressor and subsequent degradation by the proteasome.[10,11] When oxygen levels are reduced or cofactors such as iron ions are not available, PHD activity is inhibited resulting in increased HIF-α levels. Under these conditions, HIF-α translocates to the nucleus and transactivates its target genes.

In addition to hypoxia, other stimuli also result in the induction of HIF-α.[12-14] Specifically, the HIF-1α gene is under the control of NFκB[13,15,16] and the chromatin remodelling complex SWI/SNF.[17] Furthermore, NFκB also controls HIF-1β directly and HIF-2α indirectly,[14] making NFκB a key regulator of the HIF system. NFκB is the collective name for a family of important transcription factors that control many cellular processes such as apoptosis and proliferation (reviewed in ref. 18).

We recently reported functional crosstalk between HIF-1α and APC at

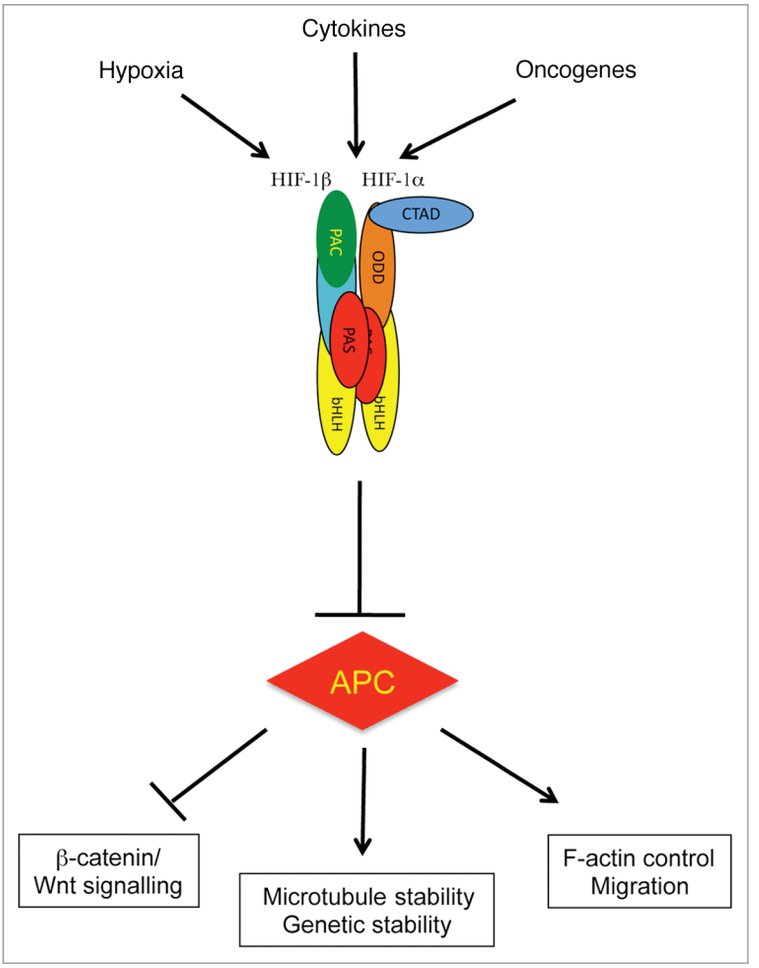

Figure 1. HIF-1α represses APC. Schematic diagram depicting how hypoxia, cytokines and oncogenes induce HIF to produce transcriptional repression of APC and hence deregulation of APC function.

the transcriptional level;[19] depletion of HIF-1α results in increased APC mRNA and protein, just as depletion of APC results in increased HIF-1α. The former is the result of direct transcriptional repression of APC by HIF-1α. We discovered a hypoxia-responsive element (HRE) in the APC promoter and demonstrated that hypoxia induces HIF-1α binding to this site. Importantly, hypoxia promotes a reduction in APC mRNA and protein in a variety of cells, suggesting that suppression of APC by hypoxia can contribute to increased survival in hypoxic conditions in tumors with wild-type APC.

Cytokines and oncogenes can induce HIF levels and activity[12,14,20] suggesting that these stimuli can modulate APC levels via HIF-dependent mechanisms (**Fig. 1**). Cytokine- and oncogene-induced repression of APC is predicted to increase β-catenin and Wnt signaling, allowing cells to progress to a more proliferative phenotype (**Fig. 1**).

Our study demonstrated that HIF-1 represses the APC promoter directly and does not discriminate between wild-type and mutant APC. Consistently, hypoxia results in decreased levels of mutant (truncated) APC protein in cancer cells. The significance of this observation is not clear. Truncated N-terminal fragments of APC can interact with a number of proteins.[7] The activity of these fragments may be different in isolation than in the context of the full-length molecule. For instance, N-terminal fragments are more active in stimulating the GEF activity of ASEF than full-length APC.[21,22] Furthermore, N-terminal domains of APC bind to C-terminal regions, which are lacking in tumor cells.[23] This interaction can regulate protein interactions of the N-terminal domain.[23] Thus in tumor cells, when the C-terminal region is missing, such regulation is not available, leaving the isolated N-terminal fragments unregulated.[23,24] The exact nature of the functions or activities carried out by N-terminal fragments is not clear at all. However, expression of N-terminal APC fragments in diverse cells and tissues has strong dominant effects on cell migration (unpublished observations). At this time, the direct consequences of the loss of truncated APC fragments induced by elevated HIF-1α are not understood and require further studies.

A reciprocal repression of HIF-1α by APC was also observed. APC depletion results in increased HIF-1α levels and activity (**Fig. 2**). This increase is mediated by NFκB and requires regulation of β-catenin by APC. In this case, the level of active β-catenin seems to be crucial for the ability of APC to regulate HIF. This is likely related to the fact that, while too much β-catenin activity results in NFκB inhibition, low levels of β-catenin can promote NFκB activity to induce HIF-1α (**Fig. 2**). This observation is in agreement with other studies that have suggested a dose-dependent effect of β-catenin in producing a malignant phenotype in cancer cells.[25]

Our results suggest that cells lacking APC are adapted to hypoxia and hence have a survival advantage under hypoxic conditions. This could be an important factor in the progression of colorectal tumors even at the early stages.

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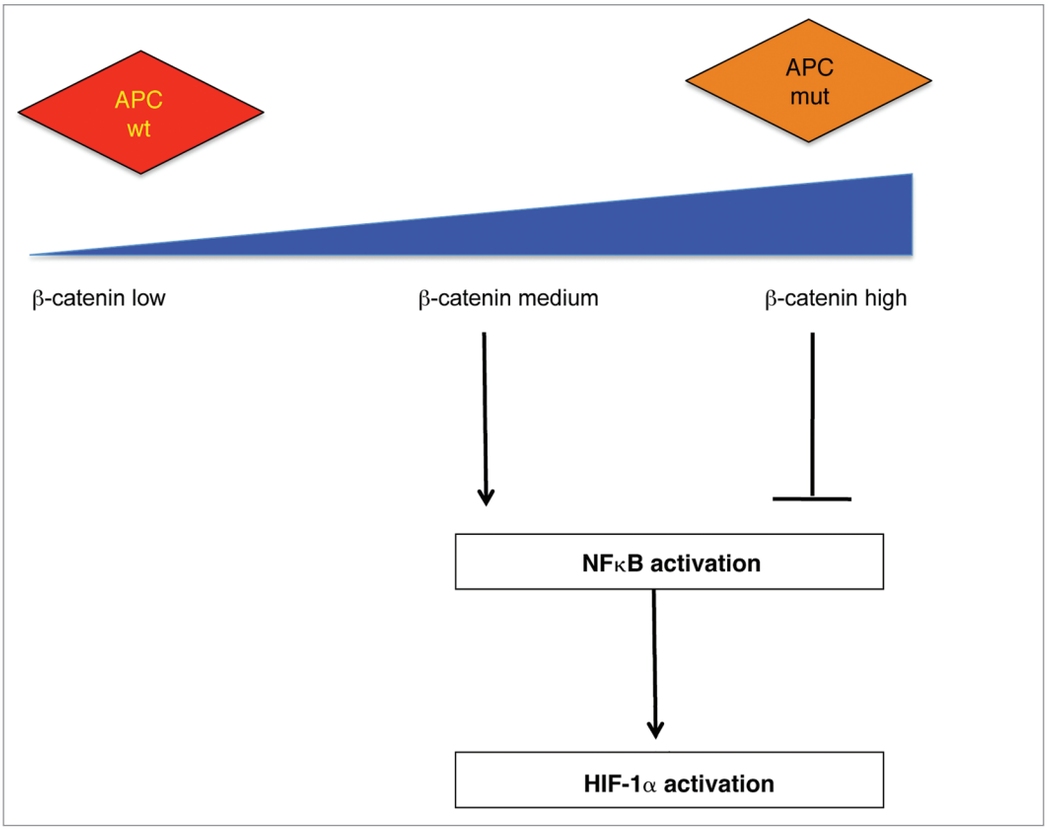

Figure 2. APC represses HIF-1α. APC control of HIF-1α is indirect and requires wild-type APC, low levels of β-catenin and NFκB activity. Mutations in APC result in high levels of β-catenin, which inhibits NFκB, and hence suppresses changes in HIF-1α.

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