cells. In contrast, MEFs that lacked Wip1, p19 and p16 were fully oncogenic when the oncogenes *Hras* or *Myc* were introduced. MEFs that lacked only Wip1 and p16 had an intermediate phenotype, as they were fully oncogenic when transformed by certain combinations of oncogenes, but not by others. The conclusion was that loss of Wip1 activates two tumor suppressor pathways, p19 and p16, both of which contribute to the resistance to transformation of cells lacking Wip1.

Living proof

Encouraged by the *in vitro* data, Bulavin *et al.*[3] went on to ask if ablation of *Ppm1d* would inhibit oncogenesis *in vivo*. The authors decided to study breast cancer development, as Wip1-null mice have a strong induction of p16 in the normal mammary epithelium, and previous work indicated that the presence of cyclin D1 (whose action is inhibited by p16) is required for induction of breast cancer by certain oncogenes[13]. The authors crossed mice deficient for Wip1 with three different strains of mice, each engineered to overexpress a different oncogene in the epithelium of the mammary gland. Bulavin *et al.*[3] found that mammary tumorigenesis in mice that expressed the *Wnt1* oncogene in the mammary gland was not affected by the absence of Wip1, whereas mice that expressed either *Hras* or *Erbb2* in the breast epithelium were relatively resistant to the development of breast cancer in the absence of Wip1. Finally, the authors show that when treated with a specific p38 MAPK inhibitor, tumor-resistant Wip1-null mice that express *Erbb2* in the breast repressed expression of p16 and developed breast tumors. Together, these data indicate that the absence of Wip1 prevents breast cancer induction through constitutive activation of p38 MAPK, which in turn causes upregulation of the tumor suppressor p16 (**Fig. 1**).

Wip1 as a drug target

The present study indicates that inhibition of the Wip1 phosphatase could suppress the proliferation of certain types of cancer, most notably breast cancer. Phosphatases are, in principle, susceptible to targeting by drugs, as potent inhibitors of other phosphatases have been developed. The side effects of inhibition of Wip1 may also be acceptable, as Wip1-null mice develop normally, even though defects in immune function have been noted[12]. Not all types of cancer respond to antiproliferative signaling through the p16 or p19 tumor-suppressor pathways. Indeed, Bulavin *et al.*[3] show that breast cancers caused by the *Wnt1* oncogene in mice are not inhibited by loss of Wip1. But a substantial fraction of breast cancers have increased expression of cyclin D1, and such tumors may benefit from Wip1 inhibition[14].

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Shutting down Wnt signal–activated cancer

M Mark Taketo

New evidence suggests that Wnt signaling can be suppressed or further activated by upstream signals, even though the pathway seems to be constitutively activated by downstream mutations in cancer cells.

The Wnt signal pathways have key roles in embryonic development. Defects in the pathway have also been implicated in cancer of the colon and other organs[1,2]. Two Wnt pathways have been identified: the canonical and noncanonical pathways. Activation of the canonical pathway induces transcription of a new set of genes through the β-catenin–T cell factor (TCF) complex, which regulates cell proliferation and differentiation[1]. Activation of the noncanonical pathway does not require β-catenin signaling and controls cell movement during morphogenesis[2].

In the absence of Wnt ligands bound to their receptors, the cytoplasmic complex of APC and Axin provide a scaffold for GSK3β to phosphorylate β-catenin (**Fig. 1a**). Phosphorylated β-catenin is then rapidly degraded through the ubiquitin pathway. When Wnt ligands bind to the cell-surface receptor Frizzled (Fzd), they trigger the phosphorylation of a cytoplasmic effector, Dishevelled (Dsh), which then inhibits the activity of GSK3β on the APC-Axin complex. Unphosphorylated, and therefore stable, β-catenin can then accumulate in the cytoplasm and form a complex with TCF in the nucleus, which initiates transcription of Wnt target genes (**Fig. 1b**).

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Canonical Wnt signaling in cancer

Most colon cancers and other digestive cancers are associated with mutations in *APC*, *AXIN1* or *CTNNB1*, and ~90% of colon cancers are associated with defects in the canonical Wnt signaling pathway (**Fig. 1c**)[1]. Mutant APC and Axin are unable to assist GSK3β in phosphorylating β-catenin. Similarly, mutations that lead to amino acid substitutions in the phosphorylated residues of β-catenin stabilize the protein. Either type of disruption causes constitutive signaling independent of the upstream signal from Wnt.

On page 417–422, Hiromu Suzuki and colleagues add a new twist to this simplistic view on the canonical Wnt pathway[3]. In an earlier paper, they isolated genes that were preferentially hypermethylated in human colon and gastric cancers[4]. Among them, they identified a family of secreted Fzd-related proteins (SFRPs) that can compete with Fzd for the Wnt ligands. Now, the authors report on experiments in which they expressed SFRPs in colon cancer cell lines carrying mutations in *CTNNB1* or *APC*. SFRP1, SFRP2 and SFRP5 suppressed Wnt-dependent transcription by ~60% (**Fig. 1d**). They then expressed *WNT1* in the β-catenin mutant cell line HCT116. Wnt pathway–dependent transcription was ~3 times

greater and was suppressed by cotransfection with constructs expressing SFRPs. Furthermore, Wnt-dependent transcription was 3.5 and 7 times greater when the authors coexpressed wild-type or stable mutant β-catenin, respectively, in these cells.

The results establish that Wnt signal activation by mutant β-catenin or APC can be partially suppressed by upstream ligand competitors, and that Wnt signaling can be boosted further in a β-catenin- or APC-mutant cell line by upstream Wnt ligands or by additional expression of β-catenin (**Fig. 1d** and **c**, respectively). Accordingly, it may be possible to suppress the tumor phenotype in Wnt-activated cancer cells by inhibiting the Fzd receptor through competition with antagonists. In fact, Hiromu Suzuki and colleagues show that colon cancer cells that overexpress SFRPs have less colony formation and a higher rate of apoptosis.

How much Wnt is required for tumorigenesis?

The authors suggest that β-catenin mutated at Ser45 in the HCT116 cells only partially stabilizes the protein, because phosphorylation may take place at three other residues, consistent with a recent report[5]. But phosphorylation at Ser45 seems to be essential for phosphorylation of the other residues[6,7]. In this regard, it would be helpful to determine if deleting the remaining wild-type allele[8], or all four phosphorylated residues[9], would have a substantial effect.

Looking at the bigger picture of the Wnt signaling system, we must consider what might be allowing this dynamic signal regulation. Expression of exogenously introduced wild-type and Ser45-mutant β-catenin increased Wnt signaling in HCT116. Thus, Wnt signal–dependent transcription is not saturated in cells that carry the Ser45 mutation in only one of the alleles[10]. The increased Wnt signaling by expression of extra ligand can also be explained by Dsh-dependent inhibition of GSK3β phosphorylation of wild-type β-catenin synthesized from the other allele. Regarding SW480 cells with biallelic *APC* mutations[11], additional signal from Wnt ligands may be explained by the flexible nature of the APC-Axin complex. For example, overexpression of Axin can compensate for the lack of APC in phosphorylating β-catenin[12]. Dsh inhibits GSK3β phosphorylation of β-catenin on the APC-Axin scaffold, but its mode of inhibition is not fully understood. Slowly, the pieces of the Wnt signaling puzzle are coming together and suggest that signaling can be regulated at many levels in a quantitative manner, with a wide, dynamic range.

Approximately 90% of primary colon cancer tissues showed methylation of *SFRP1*, whereas only ~70% had *APC* mutations. Notably, methylation of *SFRP1*, *SFRP2* and *SFRP5* was also found in ~90% of early colonic adenomas. Accordingly, silencing SFRP genes may be one of the earliest events in tumorigenesis. The new results suggest that we may be able to suppress Wnt signaling in cancer even when *APC* or *CTNNB1* is mutated.

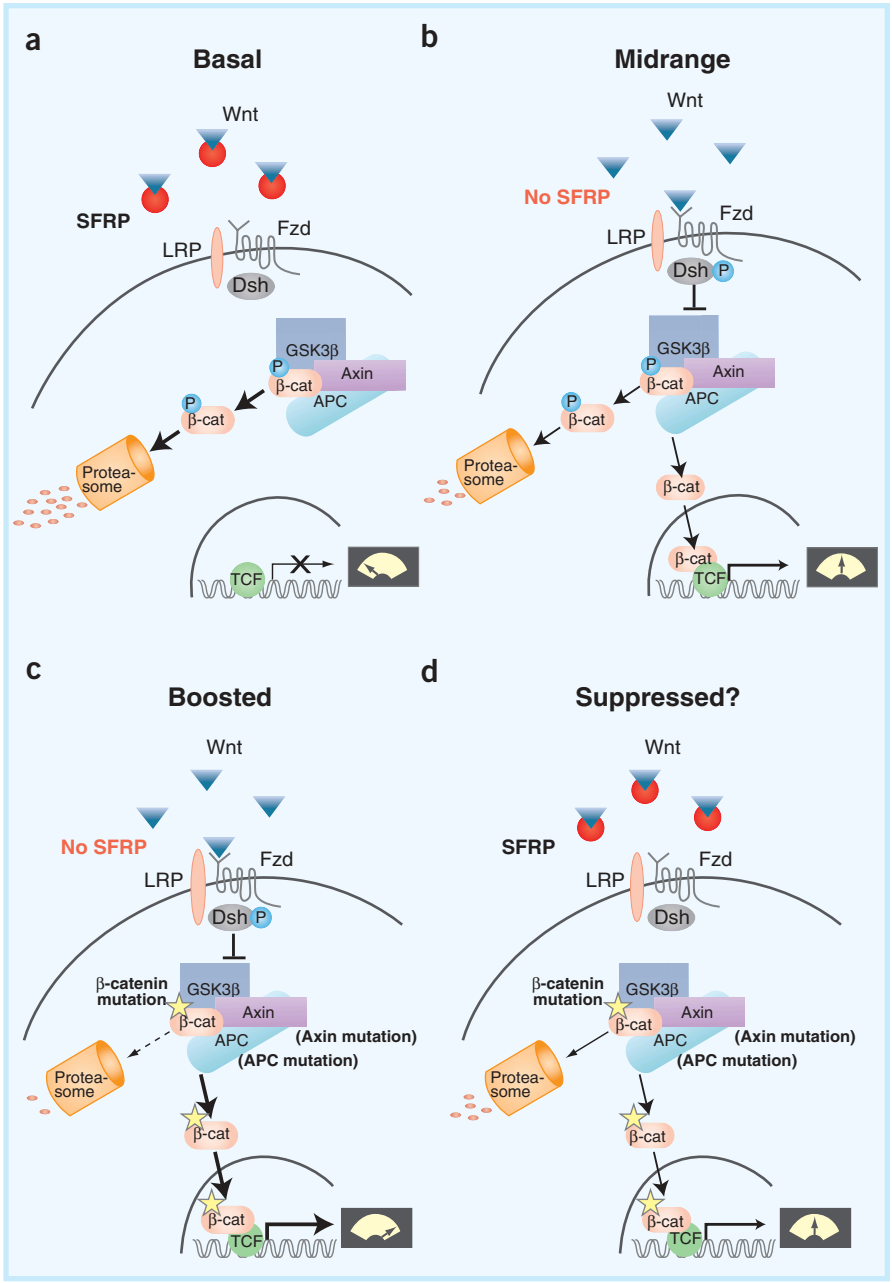

Figure 1 Quantitative control of the canonical Wnt pathway. (**a**) Basal level. In the absence of Wnt ligand binding to Fzd receptors, most β-catenin molecules are rapidly phosphorylated by GSK3β in the APC-Axin scaffold and degraded through the ubiquitin pathway. (**b**) Midrange activation. When SFRPs are reduced by promoter methylation, Wnt ligands bind to Fzd and induce phosphorylation of Dsh, which in turn inhibits GSK3β on the complex. A sizable fraction of β-catenin remains unphosphorylated and binds to TCF in the nucleus. (**c**) Boosted activation. In addition to Wnt ligand activation of Fzd, downstream effector β-catenin carries a stabilizing mutation. A much larger fraction of β-catenin remains unphosphorylated and induces strong transcription of target genes. Mutations in *APC* or *AXIN1* can cause similar effects. (**d**) Suppressed activation. SFRPs trap Wnt ligands, which partially suppresses signaling, even when β-catenin carries a stabilizing mutation. LRP, co-receptor, low-density-lipoprotein receptor–related protein family[13].

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USF1 on trial

Carol C Shoulders

The ubiquitous USF proteins regulate the transcription of many genes involved in lipid and glucose homeostasis. A new study provides genetic evidence that USF1 may confer susceptibility to high blood lipid levels.

Familial combined hyperlipidemia (FCHL), the most common inherited disorder of abnormal blood lipid levels, is believed to share part of its etiology with the metabolic syndrome[1]. The extent of this overlap has been difficult to define because of important differences in diagnosis. FCHL is typically diagnosed through an index individual affected with marked hyperlipidemia (elevated blood cholesterol or triglyceride levels) and normally excludes individuals with hyperlipidemia secondary to other coronary heart disease (CHD) risk factors, such as central obesity and maturity-onset diabetes (type 2 diabetes). More fundamentally, a diagnosis of FCHL demands that index individuals have a blood relative with primary hyperlipidemia, whereas the metabolic syndrome does not. On page 371–376 of this issue, Päivi Pajukanta and colleagues[2] present evidence that a specific haplotype of the gene encoding upstream transcription factor 1 (*USF1*) located in the chromosome 1q21–23 region, is associated with FCHL. They propose that *USF1* is a good candidate for conferring susceptibility to the core features of the metabolic syndrome: insulin resistance, glucose intolerance, type 2 diabetes, central obesity, dyslipidemia (elevated blood triglyceride or low high density lipoprotein-cholesterol) and hypertension[2].

Homing in on USF1

Pajukanta *et al.*[3] previously found evidence for linkage of the chromosome 1q21–23 interval to FCHL in a cohort of 31 Finnish families, identified through an index individual with early-onset CHD and blood cholesterol or triglyceride levels greater than or equal to age- and sex-specific 90th percentiles. Subsequent studies supported these data and implicated the same chromosomal region in the etiology of type 2 diabetes[4–6].

In the present study, Pajukanta *et al.*[2] built on their previous work by systematically examining a stretch of DNA containing about 10 Mb for a genetic lesion conferring susceptibility to FCHL[2]. This involved tracking the transmission of 56 functionally unbiased single-nucleotide polymorphisms (SNPs) to the affected family members of 42 pedigrees with FCHL, including the 31 families who participated in the original linkage study. They examined six SNPs that had some association with FCHL in an additional 18 extended families. The two SNPs that produced the best evidence for association with FCHL are both located in the gene encoding USF1, a transcriptional activator that regulates a number of genes involved in whole-body lipid and glucose homeostasis[7,8]. This report prompts two important questions. First, how compelling is the genetic evidence for the involvement of *USF1* in FCHL and, by implication, the etiology of the condition? Second, irrespective of the contribution of *USF1* to FCHL, do specific *USF1* haplotypes increase the risk of type 2 diabetes or the metabolic syndrome?

Regarding the first issue, in the absence of existing linkage data, some investigators[9] argue for a statistical criterion of $P < 5 \quad 10^{-9}$, whereas others emphasize the importance of replicating results in independent data sets and of biological plausibility[10]. In the present FCHL study, the alleles at the two associating loci (*usf1s1* (exon 11) and *usf1s2* (intron 7)) are in strong linkage disequilibrium and, based on family data, have major allele frequencies of about 0.65 in the Finnish population. The lowest *P* value ($9 \quad 10^{-7}$) was obtained for the triglyceride trait in men using the gamete-competition test, which views the transmission of alleles or haplotypes to affected individuals as a contest. These data were supported by the results of a second test statistic (HBAT-o) that determined which specific haplotypes were preferentially transmitted. The haplotype containing the minor alleles at the *usf1s1* and *usfs2* loci was transmitted less frequently to affected males with the triglyceride trait ($P = 4 \quad 10^{-3}$), prompting the authors to suggest that this might protect against FCHL in their families.

The HBAT-o test also produced evidence ($P = 7 \quad 10^{-4}$) for preferential transmission of the common *USF1* haplotype to males with the triglyceride trait of FCHL, supporting the results from three different analyses: the gamete-competition test; a multilocus geno-PDT and a haplotype-based haplotype relative risk test. But even robust statistical data from a single cohort of families are not always replicated in another population. Because the Finnish families were, necessarily, selected on the basis of a complex phenotype and did not exclude individuals with type 2 diabetes, the size of the effect of the putative *USF1* risk haplotype on any single FCHL-related trait is uncertain. Given the relatively high frequency of the risk haplotype, this effect will presumably vary according to an individual's susceptibility to develop FCHL, which we now know is attributable to at least four other genes[11].

A SNP in an internal promoter

The evidence for association of *USF1* with FCHL prompted Pajukanta *et al.*[2] to sequence this gene in 31 FCHL probands[2]. They found no amino acid changes in USF1 that could account for association of the putative *USF1* risk haplotype with FCHL. Nor could they find any difference in *USF1* mRNA levels in fat biopsy samples from individuals with FCHL with the risk haplotype versus those without. They did, however, identify a

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