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CDC4/FBXW7 and the ‘just enough’ model of tumourigenesis

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Abstract

There is good evidence to show that cancer-causing mutations are not always simple gain- and loss-of-function changes. One example is the *APC* gene, where the combination of mutations produces a ‘just-right’ level of Wnt signalling. A recent article by Berger and colleagues posited a ‘continuum model’ in which increasing or decreasing gene expression of function was linearly associated with tumourigenesis. Berger also proposed an ‘obligate haploinsufficiency’ or ‘fail-safe’ model, whereby heterozygous mutations produce sufficient derangement for tumourigenesis, yet homozygous mutations are cell-lethal or senescence-causing. One gene highlighted by Berger and colleagues as an example of a gene following a ‘continuum’ or ‘fail-safe’ model was *FBXW7/CDC4*, a gene mutated in several different types of malignancy. We have analysed the COSMIC *FBXW7* data. *FBXW7* does not obviously follow a ‘continuum’ or ‘fail-safe’ model and the most common mutant genotypes are mono-allelic missense changes that affect critical arginine residues involved in interactions with substrates. There is no strong selection for complete loss of *FBXW7* protein function, but bi-allelic inactivating mutations do occur. For *FBXW7*, we suggest a variant of ‘just right’ which we call ‘just enough’. For *FBXW7* mutations that occur away from the propellor tips, the heterozygote may have some effect on tumourigenesis, but there is selective pressure for a ‘second hit’. For propellor tip mutations, by contrast, there is weak pressure for a ‘second hit’ because they usually provide sufficient functional derangement on their own.

Keywords

continuum model; just right model; just enough model; two hits; *FBXW7/CDC4*

The identification of mutant genes that drive tumourigenesis has relied on two factors: an observed mutation frequency above background and an ability to assign a strong effect on protein function. For most of the established cancer genes in human tumours, mutations either inactivate protein function in the fashion of a classic tumour suppressor or activate oncogene function by mutation of specific amino acids or by another mechanism, such as translocation or copy number change. Thus, whilst not all mutations of a gene necessarily have identical effects, all *VHL* mutations are thought to inactivate the protein and all *KRAS*, *NRAS*, and *BRAF* mutations cause constitutive activity.

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For some time, however, there has been gradually increasing evidence that the simple gain- and loss-of-function models do not always apply. These debates go back a relatively long way. There is, for example, still discussion as to whether some p53 mutations result in gain of protein function and, if so, whether this is important for tumour growth [1]. Another example of evidence against a simple loss-of-function model has come from the *APC* (adenomatous polyposis coli) gene, a tumour suppressor in colorectal cancer. Bi-allelic ‘null’ *APC* mutations are rarely found in human tumours. Instead, the ‘two hits’ are co-selected to leave a critical, yet partial, ability to down-regulate β -catenin and hence lower the level of Wnt signalling. The explanation for these observations probably lies in the fact that too high a level of Wnt signalling is sub-optimal for tumourigenesis [2–4]. Indeed, different regions of the bowel have different optimal levels of Wnt for tumour growth. These selective constraints on somatic *APC* mutations have been dubbed the ‘just right’ model of tumourigenesis [5] (Figure 1).

We have previously suggested that the ‘just right’ model might also apply more generally in tumourigenesis, less in terms of the spectra of mutations in individual genes, and more as an explanation for why specific cancer types tend to acquire mutations in specific genes, sometimes within the same signalling pathways [6]. Thus, we hypothesize that *APC* may be unusual, in that different mutant alleles can have optimal or suboptimal effects, but the ‘just right’ principle may hold more widely.

A recent article by Berger *et al* [7] focused on the interesting topic of the importance of gene dosage changes in promoting tumourigenesis. In summary, the authors highlighted the possibility that decreases or increases in gene function short of full inactivation or activation—for example, through transcript level variation—might have impacts on cancer development. This is not necessarily a form of the ‘just right’ model, however, since increasing loss- or gain-of-function may be monotonically associated with increasing cancer risk.

One, more controversial, possibility that Berger *et al* highlighted [7] was a phenomenon that they called ‘obligate haploinsufficiency’, whereby heterozygous mutations produce sufficient derangement for tumourigenesis, yet homozygous mutations are cell-lethal or senescence-causing. The notion of Berger *et al* is that this provides some sort of ‘fail-safe’ mechanism, such that a ‘second hit’ causes cell lethality or senescence. This intriguing model can be criticized for its lack of theoretical underpinning, since the initial heterozygous mutation must provide a selective advantage and the growing tumour can easily tolerate the occasional cell death owing to ‘second hits’. In fact, recessive, classical tumour suppressor mutations are a much better defence against cancer than ‘fail-safe’ mutations. We suggest that the ‘fail-safe’ category should be renamed—perhaps to the ‘heterozygote advantage’ model—and contend that such mutations are found because of a requirement for an optimal level of derangement by tumour cells, rather than any sort of evolutionary advantage for the organism. Whatever its name, we regard this model as one particular form of the ‘just right’ scenario.

One gene highlighted by Berger *et al* [7] as an example of a gene following a ‘continuum’ or ‘obligate haploinsufficiency/fail-safe’ model was *FBXW7/CDC4*. This gene encodes the

substrate specific component for a ubiquitin ligase complex, the targets of which include cyclin E, c-Myc, Notch, c-Jun, and TGIF1 [8,9]. FBXW7 comprises eight WD40 repeats which encode propellor blade structures that bind the phosphodegron of ubiquitin ligase targets through arginine residues at the propellor tips. *FBXW7* is mutated in several different types of malignancy, including solid tumours and haematological neoplasms. Loss of *FBXW7* has been associated with chromosomal instability [10], but this association is not found *in vivo* and its mutation spectrum in cancers is actually rather unusual: like *APC*, there appear to be relatively few null alleles [11]. *FBXW7* does not obviously follow a ‘continuum’ or ‘fail-safe’ model. In fact, the mutations found in tumours tend to be of several types, in decreasing order of frequency:

- (i) missense changes that affect the critical arginine residues, especially those at positions 465, 479, and 505;
- (ii) missense changes elsewhere in the gene;
- (iii) nonsense mutations throughout the gene;
- (iv) frameshift mutations throughout the gene;
- (v) loss of heterozygosity (LOH), generally of unknown type and copy number.

In the first four categories, C>T changes are overwhelmingly the most common.

The COSMIC database (<http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=gene&ln=FBXW7>) lists 286 *FBXW7* mutations by position, and LOH (in the form of zygosity) is reported for about 40% of these (Figure 2). Six tumours have two reported mutations at the base-pair level. Analysis of *FBXW7* mutations by tumour site is difficult owing to the modest mutation frequency in any one type of tumour, but there are no gross differences in mutation spectrum across tumours. We have therefore analysed the COSMIC *FBXW7* data across all tumours, with the aim of using human genetic data to explain the mutation spectrum and to find out whether the ‘just right’, ‘continuum’ or ‘fail-safe’ model—or, indeed, some other model—might apply in this case.

Inspection of the data allows five initial, simple observations:

- (i) no tumours have two protein-truncating mutations, but some (9/117, 7.7%) have one protein-truncating mutation and LOH;
- (ii) the propellor tip mutations are rarely accompanied by a ‘second hit’ (7/57, 12.3%) compared with other mutations ($p = 0.008$, Fisher’s exact test; Table 1);
- (iii) owing to this, missense mutations overall have a significantly lower frequency of LOH than truncating mutations ($p = 0.041$, Fisher’s exact test; Table 2);
- (iv) most missense and non-propellor tip truncating mutations are not accompanied by LOH, and non-propellor tip missense mutations have a similar rate of ‘second hits’ (mutation or LOH) to truncating mutations (29% versus 36%, $p = 0.76$, Fisher’s exact test; Table 3);
- (v) non-propellor tip missense mutations tend to be later in the gene—generally in the propellor blades—than truncating mutations ($p = 0.044$, Wilcoxon test).

These findings suggest the following:

- (i) there is no strong selection for complete loss of FBXW7 protein function;
- (ii) propellor tip mutations can be regarded as a separate category and there is weak (if any) selective pressure for them to acquire ‘second hits’: they provide sufficient functional derangement on their own;
- (iii) non-propellor tip missense mutations probably have partial loss-of-function for substrate binding (and, of course, truncating mutations cause partial or complete loss-of-function);
- (iv) tumours with complete loss of FBXW7 function (based on bi-allelic missense or truncating mutation or LOH) exist at a non-trivial frequency (and are relatively more frequent than tumours with zero 20AARs in *APC*);
- (v) even taking account of the noisiness of LOH analysis and fact that we do not know chromosome 4 copy number for most tumour studies, there appears to be some selective pressure for second hits, especially when the first mutation is not at the propellor blade tip.

These findings are further complicated by the potential for dominant-negative (missense) mutant *FBXW7* alleles if the protein acts as a homodimer, as has been postulated. A plausible unifying explanation is that propellor tip mutations are dominant negatives and reduce functional protein levels below 50%. *In vitro* assays suggest that this is the case, but these are not decisive. For example, overexpressing propellor-mutant *FBXW7* reduces degradation of overexpressed cyclin E [12], although this might just be as a result of mutant protein binding, but not degrading, its substrate. Even extreme overexpression of propellor mutant *FBXW7* does not cause cyclin E levels close to those obtained in a null system.

Berger *et al* [7] did not make a definite pronouncement as to the model of selection of *FBXW7*, calling it both ‘haploinsufficient’ and (on the basis of mouse models) ‘dosage-dependent’. We contend that it is important to distinguish between the different *FBXW7* mutations found in human tumours. Consequently, discussion of the various mouse models needs to take into account the extent to which those mutations resemble the human situation before general conclusions can be drawn as to how deficient *FBXW7* function promotes tumour growth. Our own view is that *FBXW7* propellor tip mutations provide sufficient selective advantage on their own. For other mutations, there is pressure for two hits—or, more accurately, absence of wild-type protein, since two truncating or ‘null’ mutations are very rare and it remains possible that complete loss-of-function is disadvantageous. Many tumours without propellor tip mutations do not, however, appear to acquire ‘two hits’ at *FBXW7*; this may be an artefactual finding owing to technical issues, but it might also be that a tumour’s genetic background or environment only requires mono-allelic *FBXW7* mutation, or alternatively, subsequent mutations in other genes added to a mono-allelic mutation could provide an alternative to bi-allelic mutation.

We do not disagree with Berger *et al* [7] that some mutations in individual genes may be selected in cancers even if those mutations provide partial, yet apparently sub-optimal, selective advantages: this is their ‘continuum model’. One of many fascinating questions

being tackled by new technology is deep sequencing of cancers to determine whether there exist minor tumour sub-clones with sub-optimal mutations and whether putative selective constraints are relaxed in late-stage carcinogenesis. Furthermore, the probably small, quantitative differences caused by some common germline genetic variants have non-trivial effects on cancer development. However, we add the *caveat* to the ‘continuum model’ that selection always occurs towards the optimum and the observed apparently ‘continuum’ mutations do not occur in isolation: perhaps the microenvironment or existing mutation complement selects for partial loss- or gain-of-function and perhaps subsequent mutations affecting the same underlying process occur in different genes. By contrast, we profoundly disagree with the ‘fail-safe’ model. Overall, we believe that the scenarios described by Berger *et al* [7] are all compatible with a ‘just right’ model of tumourigenesis. For the individual case of *FBXW7*, we suggest a variant of ‘just right’ which we call ‘just enough’ (Figure 1): propellor tip mutations provide sufficient derangement, whereas the other observed mutations are selected as heterozygotes for partial loss-of-function but with pressure for more profound loss-of-function in the compound heterozygote mutant state.

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Figure 1.

Comparison between the classical tumour suppressor and haploinsufficiency models of tumourigenesis, and the continuum (dose-responsive), just right, and just enough models. The dashed line in the continuum model represents a fail-safe tumour suppressor.

Figure 2.

Locations of *FBXW7* mutations in human tumours. Functional domains of *FBXW7* are shown under the chart. D = dimerization domain; F = F-box.

Table 1

Propellor tip *FBXW7* mutations are less likely than other mutations to have a 'second hit'. Samples were assigned as having second hits if they were 'homozygous' in the COSMIC zygosity field (assumed to have LOH) or if they had two mutations in the COSMIC list. Heterozygotes were listed as such in the COSMIC zygosity field

	No second hit	Second hit	Total
Propellor tip (codons 465, 479, 505) missense mutation	50	7	57
Other mutation	38	20	58
Total	88	27	115

Table 2

The combined set of missense *FBXW7* mutations has a lower frequency of LOH than does the combined set of truncating mutations. Second hits were assigned as in Table 1. Six compound heterozygous samples with one truncating and one missense mutation were assigned as half-data points to each of the two 'second hit' categories

	No second hit	Second hit	Total
Missense mutation	68	12	80
Protein-truncating mutation	16	9	25
Total	84	21	105

Table 3

Non-propellor tip missense mutations have a similar rate of LOH to truncating mutations. Second hits were assigned as in Table 1. Four samples with one non-propellor tip missense mutation and one truncating mutation were assigned as half-data points to each of the two 'second hit' categories

	No second hit	Second hit	Total
Non-propellor tip missense mutation	17	7	24
Protein-truncating mutation	16	9	25
Total	33	16	49