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Cdh1: a master G0/G1 regulator

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Abstract

APC/C^{Cdh1} controls the G0 and G1 phases of the cell cycle. Using a conditional knockout of the Cdh1 coding gene *Fizzy-related (Fzr)*, a new study demonstrates that Cdh1 is essential for viability and that it functions as a tumour suppressor by preventing genomic instability.

The timely and unidirectional progression of the cell cycle is controlled by ubiquitin-mediated proteolysis of core components of the cell-cycle machinery. Two ubiquitin ligase complexes in particular, SCF (Skp1/CUL1/F-box protein) and APC/C (Anaphase Promoting Complex/Cyclosome), control the ubiquitination and subsequent degradation of many key cell-cycle regulators, with SCF-based ligases being active throughout the cell cycle and APC/C being active from mitosis to late G1 (refs 1,2).

APC/C regulates the metaphase to anaphase transition (through the degradation of securin and shugoshin) and mitotic exit (through mitotic cyclin degradation)^{3,4}. Eleven core subunits have been identified using genetic and biochemical approaches, and although the function of most of these components remains unknown, APC/C activity is controlled mainly through the regulated binding of two co-activators, Cdc20 and Cdh1, which target distinct substrates at specific stages during the cell cycle^{3,5}. APC/C is inactive in S phase, partially due to low levels of Cdh1 and Cdc20. In G2, Cdh1 and Cdc20 accumulate, but APC/C^{Cdh1} remains inactive until late mitosis due to inhibition by the protein Emi1 (which is eliminated in prophase) and phosphorylation by cyclin-dependent kinases (CDKs) (which inhibits Cdh1 binding to APC/C until anaphase). How APC/C^{Cdc20} is kept inactive during G2 is not clear, but, in early mitosis, the bulk of APC/C^{Cdc20} is inhibited by the mitotic spindle assembly checkpoint until all kinetochores have attached to the microtubules radiating from the spindle³. After proper spindle assembly, APC/C^{Cdc20} begins the metaphase to anaphase transition by targeting securin (activating separase) and shugoshin, allowing sister chromatids to separate, before targeting the mitotic cyclins. Subsequently, during anaphase, dephosphorylation of Cdh1 allows the formation of active APC/C^{Cdh1}, which continues to ubiquitinate mitotic cyclins along with new targets, including Cdc20, driving cells out of mitosis into G0/G1. APC/C^{Cdh1} activity persists throughout G1 until Cdh1 is inactivated at the G1 to S transition through degradation, phosphorylation and binding of Emi1.

Cdh1 is not required for mitotic exit; instead, evidence suggests that it is a master regulator of the G1 phase and the quiescent G0 phase. However, the lack of a model system has prevented a full evaluation of Cdh1 function in higher organisms. The need for a mouse model of Cdh1 function has now been addressed by Malumbres and colleagues who, on page 802 of this issue⁶, describe the conditional knockout of *Fzr*.

Supporting a role for Cdh1 in G0/G1 regulation, it has been previously shown that *CDH1* mutant yeast fail to arrest when starved of nutrients, and that *fzr* mutant flies undergo an extra embryonic epidermal cell division. Additionally, Cdh1 is not expressed during the early cell divisions of embryogenesis (which lack a G1 or G2 phase), with later expression coincident with acquisition of the G1 phase^{3,7}. Furthermore, inactivation of APC/C by conditional deletion of *Apc2* in mouse livers causes quiescent, G0 hepatocytes to re-enter the cell cycle⁸. Cdh1 cooperates with CDK inhibitors and pocket proteins in maintaining the G0/G1 state. In particular, Cdh1 completes the elimination of mitotic cyclins, induces degradation of the CDK1 activator Cdc25A and targets Skp2 and Cks1 for degradation, blocking degradation of the p27 and p21 CDK inhibitors by SCF^{Skp2}. APC/C^{Cdh1} also directs the degradation of other positive regulators of cell proliferation (for example, Plk1 and Aurora A) and DNA replication (for example, Cdc6, geminin, Tk1 and Tmpk). Because Cdh1 restrains cell proliferation and is inhibited by Emi1, it has been proposed that Cdh1 functions as a tumour suppressor (Fig. 1). Finally, G0/G1 maintenance by Cdh1 has also been proposed to block post-mitotic neurons from inappropriate cycling and apoptosis, and Cdh1 has other roles in post-mitotic neurons, including control of axon growth and synapse development⁹.

Malumbres and colleagues now show that, similarly to *Fzr* inactivation in worms and flies^{7,10}, inactivation of mouse *Fzr* results in embryonic lethality (at around embryonic day 10). The lethality is due to placental insufficiency, arising from a failure of placental trophoblasts to endoreduplicate. The phenotype is consistent with known roles of Cdh1 in negatively controlling DNA replication. For example, activation of APC/C by removal of Emi1 results in degradation of geminin and cyclin A with consequent re-replication, and the levels of geminin and cyclin A are markedly increased in *Fzr*-null MEFs¹¹. Notably, the placental insufficiency can be rescued by embryo-specific deletion of *Fzr*, allowing development until parturition. However, these mice survive for only a few days and the cause of death requires further investigation.

On a cellular level, *Fzr*-null MEFs show an increase in most Cdh1 targets, although in asynchronous cells, cyclin B levels are lower, probably due to cell-cycle defects. Indeed, multiple defects are apparent in *Fzr*-null cells. Cdh1 inactivation results in faster progression from G1 to S phase but a slower overall progression through S phase, confirming previous results with RNA interference¹². As proposed by the authors, this effect is probably due to the S phase-promoting and DNA replication-inhibiting levels of CDK activity, as well as decreased loading of replication proteins onto chromatin. Additionally, *Fzr*-null cells exhibit slow and defective exit from mitosis, with a large increase in binucleated cells that fail to complete cytokinesis. However, the precise nature of this defect remains to be determined.

Accordingly, *Fzr*-null MEFs show a high degree of genomic instability. Compared with immortalized, wild type MEFs, immortalized, *Fzr*-null MEFs have more multinucleated cells, misaligned metaphase chromosomes and multipolar spindles. Consistent with *FZR* knockdown experiments, which show induction of aberrant centrosomes, micronuclei and chromosome bridges¹², *Fzr*-null MEF cultures contain large numbers of aneuploid cells that have up to 150 chromosomes and substantial chromosome aberrations. Although immortalized *Fzr*-null MEFs proliferate, they do so more slowly than their wild-type counterparts, suggesting that genomic instability impairs cell growth. Primary *Fzr*-null MEFs also show growth deficiencies and reach crisis earlier than their wild-type counterparts. The authors suggest that this effect may be due to the observed increase in p16^{INK4A} levels; however, it is also possible that the proliferation defect stems from genomic instability/DNA damage, oncogene-induced stress from CDK activation, or both. The potential induction of p19^{ARF} or the activation of DNA damage pathways were not examined, although induction of p53 and p21 has been reported following *FZR* knockdown¹².

The analysis of *Fzr* heterozygous mice by Malumbres and colleagues has provided *in vivo* evidence for the tumour suppressor and neuronal functions of Cdh1. Although these mice are less susceptible to carcinogen-induced skin tumours, by 25 months of age *Fzr* heterozygous mice show a decrease in survival, with 25% of the survivors developing epithelial tumours that are not found in wild-type mice. The other *Fzr* allele is not deleted in these tumours (other mutations cannot be excluded at present), suggesting that Cdh1 functions as a haploinsufficient tumour suppressor. Heterozygous *Fzr* mice also showed increased proliferation of cells in the subventricular zone of the brain, a region rich in neuronal stem cells, but no increase in stem cell number was detected. However, these mice showed defects in standard tests of neuromuscular vigour and coordination. The current study verifies previous models of Cdh1 function in the cell cycle, strengthens the case for Cdh1 as a tumour suppressor and validates a role for Cdh1 in the nervous system. Yet, many questions about Cdh1 function remain unanswered. It is apparent that Cdh1 loss causes genomic instability, but the precise cause of this instability is unclear. Studies in *Fzr*-null MEFs suggest that genomic instability could result from aberrant DNA replication or failures in exiting mitosis; alternatively, as reactivation of APC/C^{Cdh1} has been observed in response to DNA damage in G2, these effects may be due to a role for APC/C^{Cdh1} in DNA damage repair¹³. In addition, although conditional deletion of *Apc2* has demonstrated that APC/C is a master regulator of quiescence, the conditional knockout *Fzr* model can be used to formally demonstrate that this activity is due to APC/C^{Cdh1}.

Conditional deletion of *Fzr* will also help to address important questions about Cdh1 function in post-mitotic neurons, facilitating a true, *in vivo* assessment of the role of Cdh1 in axonal growth and patterning. Another important question is whether Cdh1 has a role in restraining the proliferation of neuronal (and perhaps other) stem cells. In this respect, Cdh1 may act similarly to FBXW7, another ubiquitin ligase adaptor that functions as a haploinsufficient tumour suppressor and regulator of haematopoietic stem cells¹⁴⁻¹⁶.

Finally, the function of Cdh1 as a tumour suppressor requires further investigation. Several studies have reported reduced expression of Cdh1 in human tumours, but *FZR* mutation has not been reported. Is *FZR* a true haploinsufficient tumour suppressor gene, or does loss of heterozygosity contribute to tumorigenesis? The remaining *Fzr* allele from tumours in *Fzr*^{+/-} mice should be sequenced, and tissue-specific knockouts can be generated to determine the effect of the loss of both alleles. Crosses with cancer-prone models (for example, *Trp53* knockouts) may be useful to decrease the latency of tumours in *Fzr*^{+/-} mice. Additionally, tumours from *Fzr* heterozygous mice should be examined to determine what additional mutations are required to support tumorigenesis.

Cdh1 regulates complex processes at both the cellular and organismal level. By generating a conditional *Fzr* mouse model, Malumbres and colleagues have provided an essential tool with which to further investigate the complexities of Cdh1 function.

References

1. Reed SI. Nature Rev. Mol. Cell Biol 2003;4:855–864. [PubMed: 14625536]
2. Nakayama KI, Nakayama K. Nature Rev. Cancer 2006;6:369–381. [PubMed: 16633365]
3. Peters JM. Nature Rev. Mol. Cell Biol 2006;4:644–656. [PubMed: 16896351]
4. Dai W, Wang X. Cell Div 2006;1:9. [PubMed: 16759372]
5. Acquaviva C, Pines J. J. Cell Sci 2006;119:2401–2404. [PubMed: 16763193]
6. García-Higuera, et al. Nature Cell Biol 2008;10:802–811. [PubMed: 18552834]
7. Jacobs H, Richter D, Venkatesh T, Lehner C. Curr. Biol 2002;12:1435–1441. [PubMed: 12194827]
8. Wirth KG, et al. Genes Dev 2004;18:88–98. [PubMed: 14724179]
9. Kim AH, Bonni A. Mol. Cell Neurosci 2007;34:281–287. [PubMed: 17223572]

10. Fay DS, Keenan S, Han M. *Genes Dev* 2002;16:503–517. [PubMed: 11850412]
11. Sivaprasad U, Machida YJ, Dutta A. *Cell Div* 2007;2:8. [PubMed: 17319958]
12. Engelbert D, Schnersch D, Baumgarten A, Wasch R. *Oncogene* 2008;27:907–917. [PubMed: 17700535]
13. Sudo T, et al. *EMBO J* 2001;20:6499–6508. [PubMed: 11707420]
14. Matsuoka S, et al. *Genes Dev* 2008;22:986–991. [PubMed: 18367647]
15. Mao JH, et al. *Nature* 2004;432:775–779. [PubMed: 15592418]
16. Thompson BJ, et al. *J. Exp. Med.* 2008

Figure 1.

Emi1 and cyclin/CDK complexes have pro-proliferative/oncogenic effects, partially through the inhibition of Cdh1 activity. To establish and maintain the G0/G1 state, Cdh1 negatively regulates DNA replication and cell proliferation through the targeted ubiquitination and subsequent degradation of multiple targets (see indicated examples). Cdh1 is also re-activated following DNA damage in G2 and may be required for the DNA damage response. Malumbres and colleagues demonstrate that Cdh1 functions as a haploinsufficient tumour suppressor, suggesting that the misregulation of each or some of these substrates/processes may lead to genomic instability and tumorigenesis.