All about E

Chromosome instability

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CDC4 was identified in 2001 as an E3 ubiquitin ligase that targets cyclin E for degradation. The fact that it is mutated in several cancer cell lines helps explain why cyclin E is upregulated in some cancers, but what is the consequence of these events in cancer cells? Christoph Lengauer and colleagues investigated this in colorectal cancer and found that loss of *CDC4* causes chromosomal instability.illustration

They began by sequencing the gene from 190 colorectal cancer samples and found that 22 contained somatic mutations. They were present at all stages of colorectal cancer development — indeed, 4/58 adenomas, which progress to malignancy only after 10–20 years, also contained mutations in *CDC4*. The defect can therefore occur very early in tumorigenesis. Some of the mutations were nonsense mutations, but all are predicted to truncate the protein within the WD40 repeats, which would impact on its ability to bind cyclin E.

So, what are the effects of these mutations in colorectal cancer cells and how might they contribute to tumorigenesis? Cyclin E regulates the G1–S transition, so an increase in its levels could increase proliferation; however, studies have also implicated upregulated cyclin E in chromosome instability. The authors disrupted both alleles of *CDC4* in the karyotypically stable colorectal cancer cell lines HCT116 and DLD-1. Both cell lines expressed increased levels of cyclin E, but grew normally. Many cells displayed other abnormalities though, such as atypical nuclei (including micronuclei) and multipolar spindles. These phenotypes correlated with aberrant mitoses — 31% were not executed correctly.

Fluorescence *in situ* hybridization using centromeric probes of several chromosomes revealed that loss of both *CDC4* alleles frequently resulted in deviations from the normal chromosome complement — chromosome instability. To confirm that this was directly attributable to loss of *CDC4*, rather than the accumulation of different mutations in the cell lines, the authors used RNA interference (RNAi) to transiently knockdown gene expression of *CDC4*. These cells accumulated cyclin E and showed an increase in micronuclei formation — used as a marker of the *CDC4*-null phenotype.

Cyclin E is the only known substrate of the ubiquitin-ligase activity of CDC4, so is upregulation of cyclin E necessary and sufficient for the *CDC4*-null phenotype? The overexpression of cyclin E causes an increase in the formation of micronuclei in the presence of CDC4 and its knockdown by RNAi prevents the formation of micronuclei in the absence of CDC4, confirming that cyclin E is the sole mediator of the effects of CDC4.

As loss of CDC4 can cause chromosome instability and can be mutated at an early stage of tumorigenesis, these results support the view that it could have a causal role in cancer formation.

ORIGINAL RESEARCH PAPER

• Rajagopalan, H. et al. Inactivation of *hCDC4* can cause chromosomal instability. Nature 428, 77–80 (2004)