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Synthetic Lethal Genetic Interactions That Decrease Somatic Cell Proliferation in *Caenorhabditis elegans* Identify the Alternative RFC^{CTF18} as a Candidate Cancer Drug Target

Jessica McLellan,*† Nigel O'Neil,* Sanja Tarailo,* Jan Stoepel,†‡ Jennifer Bryan,†§ Ann Rose,* and Philip Hieter*†‡

Departments of *Medical Genetics and §Statistics, The University of British Columbia, Vancouver, BC, Canada, V6T 1Z2; †Michael Smith Laboratories, The University of British Columbia, Vancouver, BC, Canada, V6T 1Z4; and ‡Department of Biochemistry and Molecular Biology, University of British Columbia, Life Sciences Centre, Vancouver, BC, Canada, V6T 1Z3

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Somatic mutations causing chromosome instability (CIN) in tumors can be exploited for selective killing of cancer cells by knockdown of second-site genes causing synthetic lethality. We tested and statistically validated synthetic lethal (SL) interactions between mutations in six *Saccharomyces cerevisiae* CIN genes orthologous to genes mutated in colon tumors and five additional CIN genes. To identify which SL interactions are conserved in higher organisms and represent potential chemotherapeutic targets, we developed an assay system in *Caenorhabditis elegans* to test genetic interactions causing synthetic proliferation defects in somatic cells. We made use of postembryonic RNA interference and the vulval cell lineage of *C. elegans* as a readout for somatic cell proliferation defects. We identified SL interactions between members of the cohesin complex and *CTF4*, *RAD27*, and components of the alternative RFC^{CTF18} complex. The genetic interactions tested are highly conserved between *S. cerevisiae* and *C. elegans* and suggest that the alternative RFC components *DCC1*, *CTF8*, and *CTF18* are ideal therapeutic targets because of their mild phenotype when knocked down singly in *C. elegans*. Furthermore, the *C. elegans* assay system will contribute to our knowledge of genetic interactions in a multicellular animal and is a powerful approach to identify new cancer therapeutic targets.

INTRODUCTION

Cancer cells accumulate a series of somatic mutations that ultimately contribute to tumor progression. These somatic mutations distinguish cancer cells both genetically and phenotypically from normal cells. Some mutations allow tumor cells to evade cellular checkpoints and to grow in an uncontrolled manner, whereas other mutations occur in genes required to maintain chromosomal stability (CIN) genes (Wang *et al.*, 2004; Kemp *et al.*, 2005; Yuen *et al.*, 2008). Mutations in CIN genes are thought to be an early event in tumor development (Shih *et al.*, 2001; Chin *et al.*, 2004; Vogelstein and Kinzler, 2004), predisposing cells to the accumulation of genetic changes leading to progression to a cancerous state. CIN mutations and their effect on chromosome stability may render cancer cells, relative to normal cells, sensitive to knockdown of a second gene that is required for viability only in the CIN background. This concept is known as synthetic lethality (SL) and occurs when a primary sublethal mutation (e.g., a CIN mutation) is lethal in the context of a second-site sublethal mutation (Hartwell *et al.*, 1997; Kaelin, 2005).

Recently, using a cross-species candidate gene approach, systematic gene resequencing of ~200 human orthologues of yeast CIN genes in a panel of 130 chromosomally unstable human colorectal cancers identified somatic mutations in eight genes: *CDC4, BUB1, MRE11, SMC1/SMC1L1, CSPG6/SMC3, STAG3/SCC3, NIPBL/SCC2*, and *DING/PDS1* (Wang *et al.*, 2004; Kemp *et al.*, 2005; Yuen *et al.*, 2007; Barber *et al.*, 2008). Second-site mutations that are nondetrimental to normal somatic cells, but that cause SL in combination with any of these known cancer mutations represent potential cancer therapeutic targets (Hartwell *et al.*, 1997; Kaelin, 2005; McManus *et al.*, 2009). Cross-species candidate genes for such second-site SL partners of primary CIN mutations can be derived from yeast SL genetic interaction networks and tested for conservation of SL in mammalian cell lines using a combination of siRNA and knockout mutations (McManus *et al.*, 2009). This is a relatively expensive and time-consuming task in tissue culture that involves testing the SL gene pairs singly and in combination and then assessing cell proliferation. We reasoned that *C. elegans* could provide an excellent organismal model system in which gene pairs, predicted from yeast, could be more easily directly tested in proliferating somatic tissues. Furthermore, direct screening for CIN gene–SL gene pairs relevant to cancer in a living, multicellular organism may provide additional developmental context and offers the possibility of identifying

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Address correspondence to: Philip Hieter (hieter@msl.ubc.ca).

Abbreviations used: CIN, chromosomal instability; Pvl, protruding vulva; SL, synthetic lethality; SS, synthetic slow growth.

new gene interactions given its expanded gene repertoire (~20,000 genes) compared with yeast (~6,000 genes). Furthermore, *C. elegans* also offers experimental advantages over a tissue culture system, one being the ease of gene knockdown by RNA interference (RNAi), a renewable library resource.

Systematic screening of genetic interactions causing SL has been performed in *S. cerevisiae* and to a lesser extent in *C. elegans* (Tong *et al.*, 2001, 2004; Pan *et al.*, 2004, 2006; Davierwala *et al.*, 2005; Lehner *et al.*, 2006; Ceron *et al.*, 2007; Tischler *et al.*, 2006). The ability to screen for genetic interactions causing cell lethality is more difficult in multicellular animals because many single mutations that are tolerated in somatic cells or single-celled yeast are not tolerated in the developing embryo and cause embryonic lethality. This then excludes the ability to use embryonic arrest as an indicator for a synthetic interaction in many cases, as has been done in previous studies (Lehner *et al.*, 2006). To circumvent this problem, we have developed an assay system using the somatic vulval cells of the multicellular animal *C. elegans*, where knockdown of these genes does not cause organismal lethality. We have gone on to use this system to identify genetic interactions with therapeutic relevance to colon tumors.

The *C. elegans* test system developed in this work takes advantage of the fact that cell division defects in the vulval cell lineage result in a visible postembryonic phenotype. The formation of the vulva has been extensively studied and is mediated by an EGFR/RAS/MAPK pathway that is highly conserved from *C. elegans* to humans (Sundaram, 2006). Three vulval precursor cells (VPCs) go through three mitotic cycles to form the wild-type vulva consisting of 22 cells (Sulston and Horvitz, 1977). Weidhaas *et al.* (2006) have shown that loss of vulval cells by nonapoptotic cell death results in a protruding vulva phenotype (Pvl). Treatment with radiation can cause postmitotic vulval cells to die in a stochastic manner, resulting in fewer than 22 vulval cells and the generation of abnormal vulval phenotypes, including a Pvl (Weidhaas *et al.*, 2006). We reasoned that reduced postembryonic cell division in the vulval lineage would similarly generate a Pvl phenotype and could be used as an assay for genetic interactions that cause defects in somatic cell proliferation. The screen also capitalizes on the use of postembryonic RNAi to knock down genes that may be essential for embryonic development.

We report our work validating a network of yeast synthetic lethal genetic interactions with mutations in genes found to be mutated in human colon tumor cells. We then describe the development and use of a *C. elegans* somatic cell proliferation assay to test for conservation of these interactions in the somatic cell divisions of a multicellular animal. We have identified conserved interactions between members of the cohesin complex and members of the alternative replication factor C, namely *CTF8*, *CTF18*, and *DCC1*. We have also found that *CTF4* and *RAD27* have genetic interactions with the majority of genes mutated in colon tumors. These findings posit Ctf4p, Rad27p, Ctf8p, Ctf18p, and Dcc1p as potential therapeutic targets that may selectively eliminate cancerous cells with known existing mutations in a variety of CIN genes.

MATERIALS AND METHODS

S. cerevisiae Random Spore

Double mutants between each of the six "colon tumor–implicated genes," and the five nonessential "query genes" were created and analyzed for a total of 30 double mutants. Strains used can be found in Supplemental Table S1. Heterozygous double mutant strains were first constructed by mating each of the corresponding single mutants. The haploid query gene deletion strains were disrupted with the HygB (HphMX) resistance gene in strains containing the *MATa* specific marker *can1Δ::MFA1pr-HIS3::LEU2* or *can1Δ::STE2prHIS5*$^+$ (Giaever *et al.*, 2002). Four of the colon tumor implicated genes were essential and therefore temperature-sensitive (ts) alleles were used to analyze these strains. Ts alleles were fused to a URA3 marker as described by Ben-Aroya *et al.* (2008). Knockout alleles for the two nonessential colon tumor implicated genes, *MRE11* and *BUB1*, were created by targeted disruption with the G418 resistance gene. Random spore analysis was performed as described by Tong and Boone (2006) with slight modifications. Briefly, spores from each of the double heterozygous mutant strains were plated onto haploid, single, and double selection plates. Haploid plates were Sc −his −arg −leu +canavanine (60 mg/ml) + glutamic acid (0.9 g/l), and single selection plates were additionally either +hygromycinB (300 mg/l), +G418 (200 mg/l), or −ura. Spores were diluted to an OD_{600} of 0.01–0.1 and plated at volumes of 75, 150, and 300 μl on haploid, both single selection, and double selection plates, respectively.

S. cerevisiae Growth Curves

Strains used can be found in Supplemental Table S2 and interactions tested can be found in Supplemental Figure S1. *MATa* strains containing each of the single and double mutants were isolated by sporulating the double mutant strains constructed for random spore analysis on Sc −his −leu −arg +canavanine (60 ml/l). All strains were grown overnight in YPD at 30°C except for strains for *smc1-259* analysis, which were grown at 25°C. Cultures were diluted to an OD_{600} of 0.2 in the morning, allowed to grow for 4 h in YPD, and then diluted to an OD_{600} of 0.05 in fresh YPD in 96-well plates. OD_{620} measurements were taken every 30 min after 5 min of shaking for 24 h in either a Multiskan Ascent (Thermo Fisher Scientific, Rochester, NY) or an M1000 (Tecan, Hillsborough, NC) plate reader.

Growth Curve Analysis

For each mutant analyzed there were at least three independent growth curves available for analysis. A four-parameter logistic growth model was fit to the growth curves (Pinheiro and Bates, 2000):

$$y(x) = A + \frac{B - A}{1 + \exp[(x_{mid} - x)/scal]}$$

where x is time and y is the OD reading, a proxy for cell density or population size, and A is the minimum OD reading, B is the maximum OD reading, x_{mid} is the time at which 50% of total growth is achieved, and *scal* is approximately the time taken to move from 50 to 75% of growth. We fit a mixed effects logistic growth model using the R package nlme (Pinheiro *et al.*, 2008, R Development Core Team: R Foundation for Statistical Computing, 2008; R code available upon request). To identify genetic interactions, we focused on the *scal* parameter, which is proportional to doubling time under conditions of unconstrained exponential growth. The model for *scal* had the following form: $scal_{qd} = S_{WT} + S_q + S_d + S_{qd}$, where q specified the colon implicated genes and d specified the query genes. The term S_{WT} gave the typical value of *scal* for the wild type strain and the terms S_q and S_d were the typical change in *scal* associated with a single mutation in the colon implicated genes and the query genes, respectively. The interaction term S_{qd} captured the difference between the experimental value of *scal* for the double mutant ($scal_{qd}$) and the value predicted from a simple sum of S_{WT} and the associated single mutant effects. We used this additive model of genetic neutrality to detect both synthetic and alleviating genetic interactions (see Figure 1 and Supplemental Figures S1–S3, Supplemental Table S3). A minimum definition of genetic neutrality (Mani *et al.*, 2008) was also used to analyze growth curve data and yielded similar results as the additive definition of neutrality (Supplemental Table S3, Supplemental Figure S4). Growth curve analysis for *PDS1* was not performed because the *pds1-1* and *pds1*Δ single mutants are so slow growing as to be uninformative. The interaction between *smc1-259* and *ctf8*Δ is a special case that was analyzed separately (Supplemental Figures S5 and S6, Supplemental Table S4). See Supplemental Table S5 and Supplementary Materials and Methods for further description of growth curve analysis.

C. elegans General Methods and Strains

Strains used can be found in Supplemental Table S6. All nematodes were grown at 20.0°C on standard nematode growth medium (NGM) seeded with the *Escherichia coli* strain OP50 except in cases where worms were fed *E. coli*–expressing RNAi constructs. Young adult worms were imaged on a Zeiss Axioplan 2 Imaging microscope (Thornwood, NY).

C. elegans RNAi

RNAi was administered by feeding as previously described by Fraser *et al.* (2000). All RNAi constructs, except CTF8, were obtained from the RNAi feeding library (Kamath *et al.*, 2003). A bacterial RNAi clone for *CTF8/T22C1.4* was not contained in the library and was kindly provided by K. McManus (Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB, Canada).

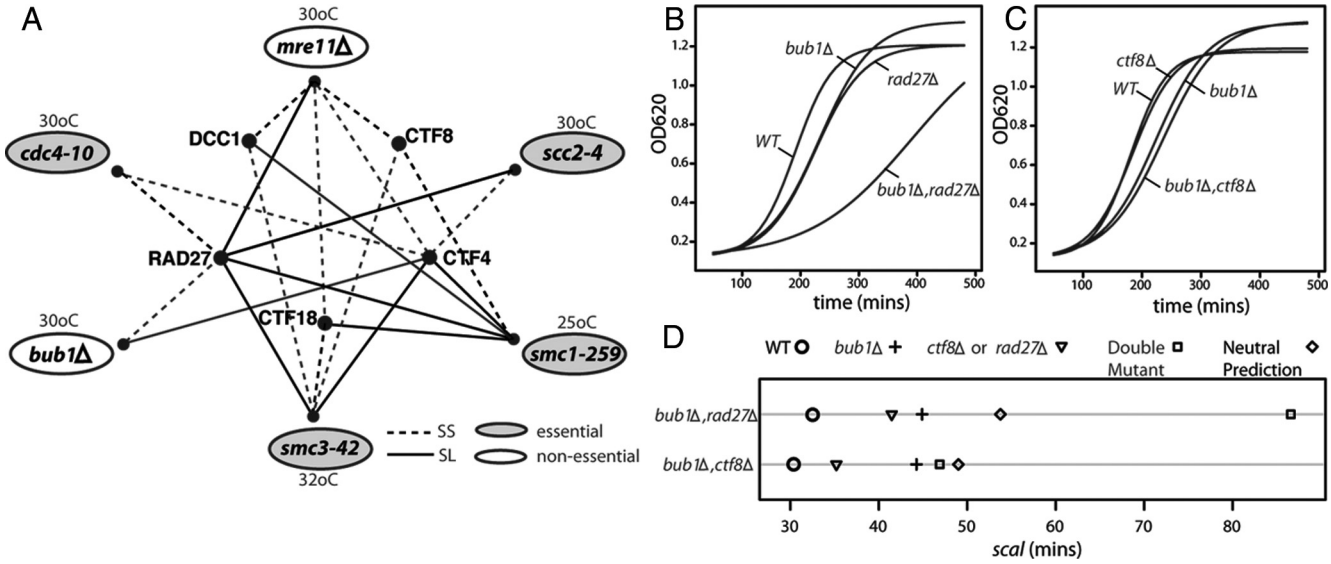

Figure 1. Synthetic genetic interactions in yeast. (A) The human orthologues of the genes on the outside of the wheel are mutated in colon tumors. Genes on the inside were identified as being SL with a subset of the genes mutated in colon tumors. Solid lines and dashed lines, SL and SS interactions, respectively. Circles shaded in gray represent essential genes in yeast. The temperature indicated next to the allele indicates the temperature at which genetic interactions were tested. (B and C) Examples of growth curves performed that yielded a SS interaction and no interaction, respectively. (D). The *scal* parameter extracted from the ensembles of growth curves in B and C are shown. The growth profile of each curve can be summarized by *scal*, a value inversely proportional to growth rate. Double mutant strains with a *scal* value statistically greater than the predicted *scal* of a neutral genetic interaction are considered SS. *scal* values for the double mutant, the associated single mutants, and the WT strain are shown on a horizontal line and slower growing strains have a greater *scal* value.

C. elegans Pvl Assay

Eight to 30 gravid hermaphrodites were alkaline lysed using 10 μl of a 1:1 solution of 2N NaOH and 10–13% sodium hypochlorite onto plates seeded with *E. coli* expressing different RNAi constructs. L1 worms hatched and then began feeding on the RNAi, allowing for all essential embryonic divisions to occur before RNAi exposure. *N2*, *him-1(e879)*, and *him-6(ok416)* adults were scored after 4 d for the presence of Pvl. *scc-1(ok1017)* and *smc-3(ok1703)* homozygotes were transferred to fresh RNAi plates after 3 d and scored for Pvl after 5 d because of slower development.

RESULTS

Genes Mutated in Colon Tumors Share Genetic Interactions with a Common Set of Genes

To further investigate the idea that a small set of genes may genetically interact with multiple genes mutated in colon tumors, we set out to complete a network of genetic interactions between these two sets of genes in yeast; we examined six of the eight CIN genes mutated in colon tumors (*CDC4, BUB1, MRE11, SMC1/SMC1L1, CSPG6/SMC3, STAG3/SCC3, NIPBL/SCC2*, and *DING/PDS1*) and five potential common "SL gene partners" (*RAD27*, *CTF4*, *CTF18*, *CTF8*, and *DCC1*). The partners were selected based on the results of several high throughput genetic interaction screens (Tong *et al*., 2004; Pan *et al*., 2006) and direct genetic tests (Mayer *et al*., 2001) that showed that *CTF18*, *CTF8*, *CTF4*, *DCC1*, and *RAD27* genetically interact with many of the yeast orthologues of genes mutated in colon tumors. We set out to complete and quantify the synthetic growth phenotypes of this network of genetic interactions by assessing all pairwise combinations of double mutants in yeast.

The genetic interactions in *S. cerevisiae* were first assayed by random spore analysis for all pairwise combinations in a 6×5 gene matrix. Nine double mutant combinations were found to be lethal, and the remaining 21 viable double mutants were further tested by growth curve analysis. Of the 21 strains analyzed 12 double mutant combinations were found to have a synthetic slow growth phenotype (SS; Figure 1, Supplemental Figures S1–S3). Quantification of genetic interactions by growth curves or other quantitative method is an important step in this process because random spore or tetrad analysis does not always clearly identify synthetic interactions. The analysis revealed that the yeast orthologues of several genes mutated in colon tumors share genetic interactions with a common set of genes, namely *RAD27*, *CTF4*, and members of the alternative RFC^{CTF18}. This analysis highlighted the importance of these five genes as potential therapeutic targets that may selectively eliminate colon cancer cells that harbor mutations in genes such as *SMC1/SMC1L1.* Scc1p is the fourth component of the cohesin complex, and although mutations in this gene were not identified in colon tumors, the hypomorphic allele *scc1-73* is also SL with deletions in *RAD27*, *CTF4*, *CTF18*, *CTF8*, and *DCC1.*

C. elegans Can Be Used to Identify Genetic Interactions in Proliferating Somatic Cells

We were interested in testing whether the genetic interactions identified in yeast were conserved in a higher organism and therefore were more likely to also be conserved in human cells. We chose *C. elegans* because it is a well-established metazoan model system with the ability to use RNAi to knock down gene function at various stages of development (Figure 2). This was important for our study because many of the genetic interactions of interest involved genes that are essential for embryogenesis, and therefore these interactions had to be studied in somatic postembryonic cells.

Our first goal was to determine whether or not the *C. elegans* vulval cell lineage could be used to identify general defects in somatic cell proliferation. To do so, we scored the frequency of individuals exhibiting a Pvl phenotype (Figure

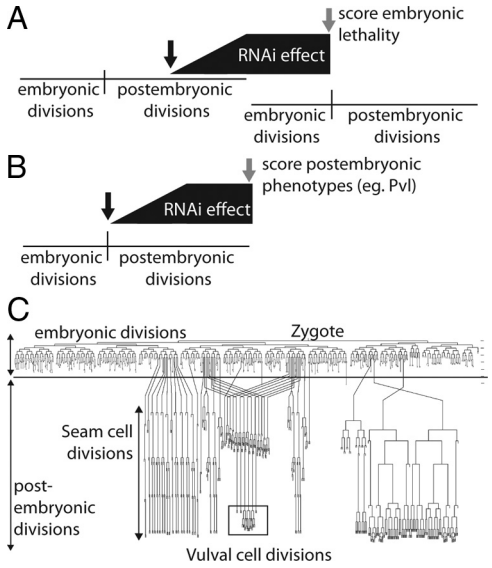

Figure 2. Paradigms for *C. elegans* genetic interaction assays. (A) Typical screening method using RNAi. RNAi treatment is initiated during the L1–L4 stages of development to deplete maternal, and hence embryonic, stores of mRNA. A frequent readout is enhanced embryonic lethality in the subsequent generation. (B) The Pvl screening method where RNAi treatment begins at the L1 stage, once animals have completed the embryonic cell divisions. This experimental approach is designed to identify phenotypes caused by defects in the postembryonic cell divisions, such as Pvl. Black arrows denote when RNAi treatment is initiated. Gray arrows indicate when phenotype scoring takes place. Black wedges depict the phenotypes caused by RNAi knockdown and the associated phenotypic lag. (C) *C. elegans* cell lineage diagram showing the embryonic and postembryonic cell divisions and highlighting the vulval and seam cell divisions (Sulston and Horvitz, 1977). Source: Adapted from the *C. elegans* server (www.umdnj.edu/mobioweb/moss/celegansonline.html/).

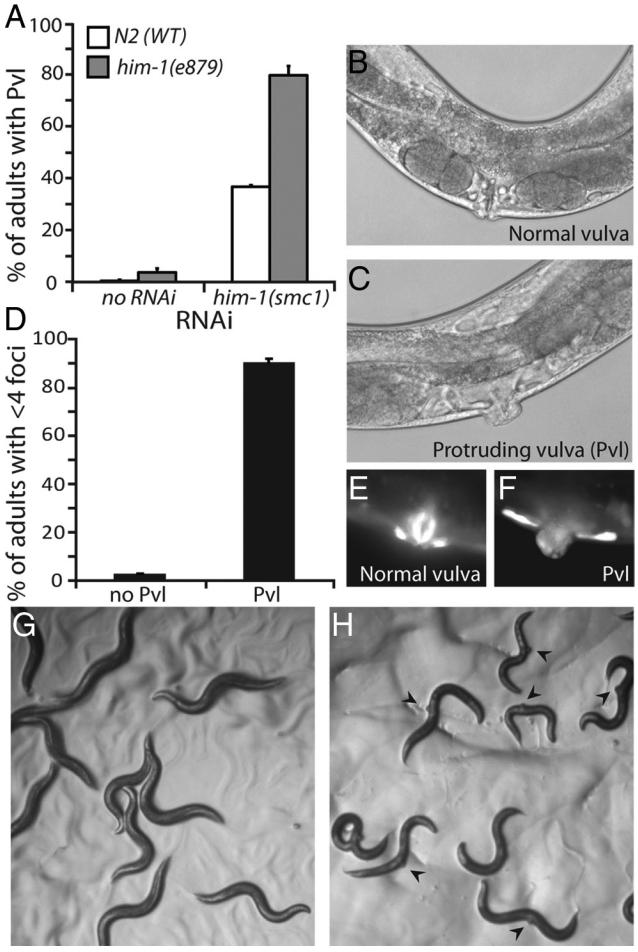

Figure 3. *him-1(RNAi)* causes Pvl. (A) N2 (wild type) worms and *him-1(e879)* worms were treated with both *him-1(RNAi)* and no RNAi and then scored for the presence of Pvl. N2 normally shows a low frequency of Pvl that is enhanced when treated with *him-1(RNAi).* The *him-1* hypomorphic mutant has a low frequency of Pvl when treated with no RNAi. Treatment of *him-1* worms with *him-1(RNAi)* produced a more penetrant phenotype (79.3 ± 3.6% compared to 3.9 ± 1.0%). Error bars, SEM; n > 500 for each experiment. (B) DIC image of N2; (no RNAi). (C) DIC image of N2; *him-1(RNAi).* (D) *zmp-1::GFP* was used to score the number of GFP-positive foci in worms with and without Pvl. Wild type typically has four GFP-positive foci, whereas 90.4 ± 1.6% of worms with a Pvl have fewer than four GFP-positive foci. Error bars, SEM; n > 120 for each experiment. (E) Image of the four GFP foci seen in worms with a wild-type vulva. (F) Image of a Pvl worm with two GFP foci. (G and H) In the Pvl screening assay worms are visually scored on a Zeiss dissecting microscope at ×160 for the presence of a small protrusion, a protruding vulva. (G) Wild-type worms treated with no RNAi. (H) *him-1* worms treated postembryonically with *him-1(RNAi).* Black arrows, protruding vulva.

3C, H) after knockdown by RNAi of the essential cohesin subunit HIM-1/Smc1p. Similar to yeast *SMC1* mutants, the hypomorphic mutation *him-1(e879)* exhibits chromosome instability (CIN), characterized by a high frequency of X chromosome nondisjunction, resulting in a high incidence of males (Him) phenotype. *him-1(e879)* also exhibits a low frequency of Pvl (3.9 ± 1.0%; Figure 3A). When RNAi targeting *him-1* was administered to the wild-type strain by feeding, after completion of embryogenesis, the percentage of Pvl in the RNAi-treated animals was 36.7 ± 1.5%. When *him-1(RNAi)* was administered to the *him-1(e879)* hypomorph, 79.3 ± 3.6% of animals were Pvl (Figure 3, A and H).

We next used the *zmp-1::GFP* marker to count a subset of cells present in the vulva in worms with a normal vulva and worms with Pvl to ascertain whether the Pvl phenotype was the result of reduced cell numbers. *zmp-1* is expressed in the two vulD and vulE cells of the vulva from L4 through adult stages. Expression is also seen in the four vulA cells in the adult stage (Inoue *et al.,* 2002). Adults have a characteristic expression pattern (Figure 3E) where the four vulA and two vulD cells are highlighted as four distinct areas of fluorescence. The vulE cells appear to have a more diffuse fluorescent signal and are visible in another plane of focus. We counted the number of total GFP-positive foci out of a total wild-type number of four in animals with and without Pvl: 90.4 ± 1.6% of animals with Pvl have fewer than four GFP foci, suggesting that these animals have fewer than the wild-type number of cells (Figure 3D). One caveat of this approach is that a change in cell morphology or expression of GFP could also result in fewer than four GFP foci. Although on its own not conclusive, a reduced number of *zmp-1* foci supports the notion that *him-1(RNAi)* results in fewer cell divisions in the vulval lineage. Collectively, these results reinforced the proposal that the Pvl phenotype could be used as an indicator of defective somatic cell proliferation.

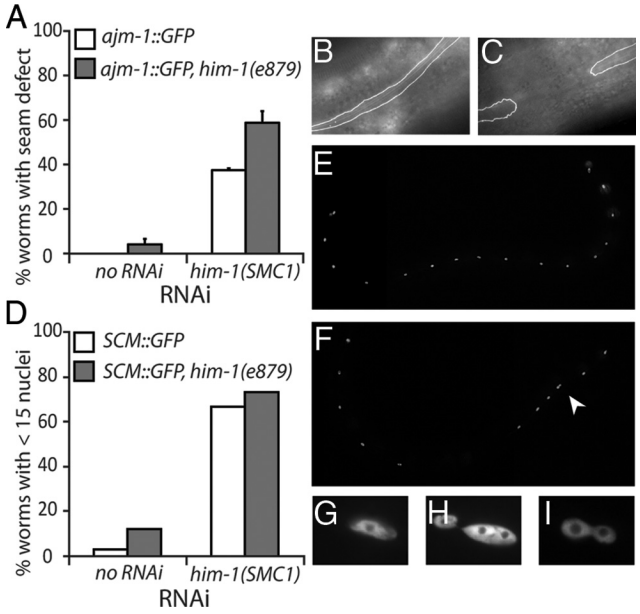

Figure 4. *him-1(RNAi)* causes defects in cell division in an independent lineage. (A) *ajm-1::GFP* worms were treated with no RNAi or *him-1(RNAi)* and adults were scored for gaps in their lateral seam syncytia. Error bars, SEM; n > 120 for each experiment. (B) *ajm-1::GFP;(no RNAi).* (C) *ajm-1::GFP; him-1(RNAi).* (D) Worms carrying the SCM::GFP marker were treated with either no RNAi or *him-1(RNAi).* (E) *SCM::GFP;(no RNAi).* 15 nuclei, not including H_0, can be identified (F) *SCM::GFP; him-1(RNAi).* Fewer than 15 nuclei are seen in 65% of worms including irregular nuclear bodies (arrowhead). (G) Example of a typical nuclear body. (H and I) Examples of irregular nuclear bodies. n = 30 for each experiment.

Defects in Division Are Seen in an Independent Postembryonic Cell Lineage

To confirm that somatic cell loss was responsible for the observed Pvl phenotype, we assayed the proliferation of an independent postembryonic cell lineage. The seam cells are a subset of cells that divide four times in the developing larva. These cells normally fuse during the L4 stage to form lateral syncytia along the A-P axis of the worm (Sulston and Horvitz, 1977; Hedgecock and White, 1985; Podbilewicz and White, 1994). We used the *ajm-1::GFP* apical junction marker (Köppen *et al.*, 2001) to visualize defects in the normally continuous lateral seam cell syncytium after postembryonic *him-1(RNAi)* treatment and found that *him-1(RNAi)* caused a statistically significant increase in seam defects in both N2 and *him-1* hypomorphic worms (Figure 4, A–C). To ensure that seam discontinuities were due to defects in cell division rather than defects in cell fusion, we used the SCM::GFP marker (seam cell marker) that marks seam cell nuclei (Terns *et al.*, 1997) to count the number of seam nuclei in adult worms. Normally adult worms have two sets of 15 seam nuclei on either side of the body, not including H_0 (Sulston and Horvitz, 1977). When N2 worms were treated postembryonically with *him-1(RNAi)*, 73% had fewer than 15 seam nuclei, and many had irregular nuclear bodies (Figure 4, D–I). These data demonstrate that discontinuities in the seam syncytium are due to defects in cell division. Thus, we conclude that the loss of *him-1* gene function responsible for the Pvl phenotype is also responsible for seam cell loss, indicating that the Pvl phenotype can be used as a general, and easily scorable, readout for proliferation defects in somatic cells.

CIN SL Genetic Interactions in Yeast Are Conserved in C. elegans

We used the protruding vulva assay to test for conservation of the synthetic genetic interactions identified in yeast, with a focus on the cohesin genes. First, we tested interactions with the *C. elegans SMC1* ortholog, *him-1*. We used *him-1(e879)*, a viable hypomorphic mutation, to screen for interactions with the *C. elegans* orthologues of *RAD27*, *CTF4*, and three members of the alternative RFC^{CTF18}. As previously mentioned, *him-1* exhibits CIN and a low frequency of Pvl (3.9 ± 1.0%). We administered postembryonic RNAi by feeding newly hatched *him-1(e879)* mutant animals to test for an increased frequency of individuals exhibiting the Pvl phenotype. Postembryonic treatment of *him-1(e879)* with RNAi against *crn-1/RAD27*, *F17C11.10/CTF4*, *Y47G6A.8/CTF18*, *T22C1.4/CTF8*, and *K09H9.2/DCC1* caused a statistically significant increase in the number of Pvl individuals (Figure 5A). Thus, we conclude that these yeast genetic interactions are conserved in *C. elegans*. It was formally possible that the increase in the Pvl phenotype observed in the *him-1* mutant was due to enhanced RNAi sensitivity in this mutant background. As part of a separate larger screen we tested the wild-type and *him-1* strains with 17 RNAi

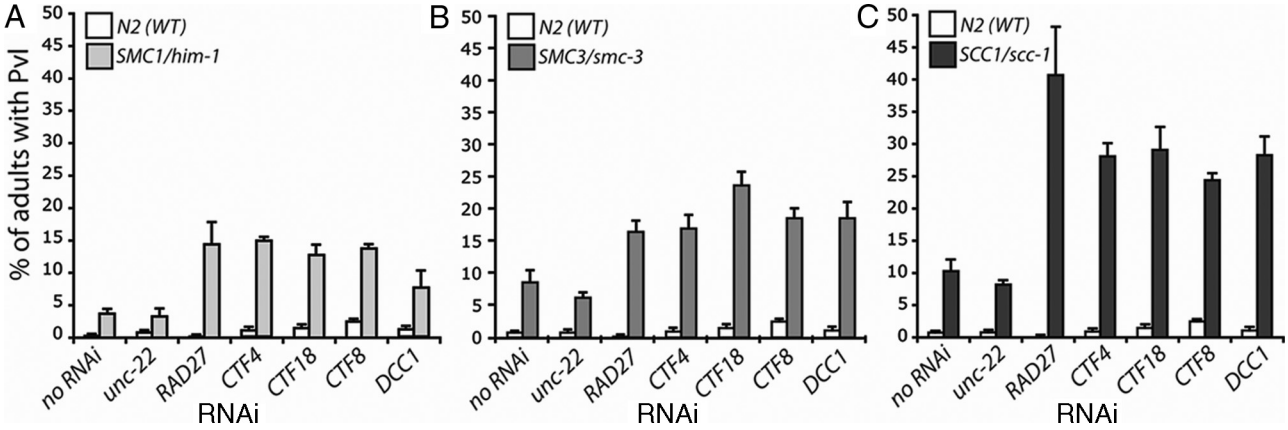

Figure 5. Genetic interactions of cohesin can be recapitulated in *C. elegans*. (A) An increase in Pvl frequency is seen when *him-1* worms are treated with RNAi against the *C. elegans* orthologues of *RAD27, CTF4, CTF18, CTF8,* and *DCC1* but not with control RNAi. (B and C) *smc-3(ok1703)* and *scc-1(ok1017)* homozygotes show a similar profile of genetic interactions. Error bars, SEM; n > 500 for each experiment.

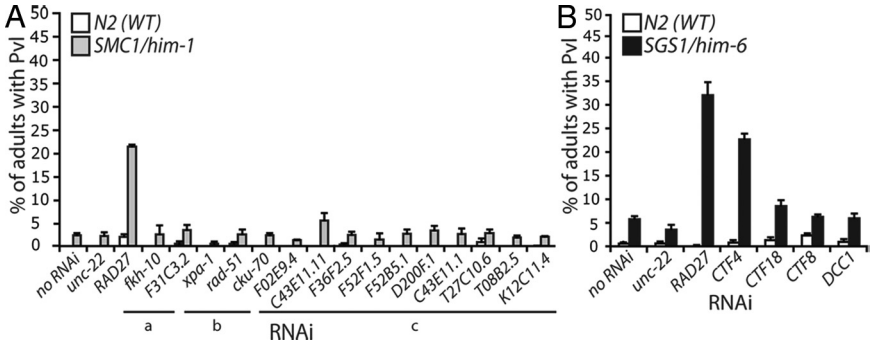

Figure 6. Genetic interactions are specific rather than general. (A) The frequency of Pvl does not synergistically increase when *him-1* is treated with randomly chosen RNAi clones. (a) RNAi clones shown to interact with the *MAD1/mdf-1* checkpoint component. (b) Genes involved in different aspects of DNA metabolism. (c) Randomly chosen genes from chromosome I. RNAi against *RAD27/crn-1* was included as a positive control. Error bars, SEM; n > 150 for each experiment. (B) *him-6* worms show a synthetic interaction when treated with RNAi against *RAD27* and *CTF4,* whereas no interaction is detected with the components of the alternative RFC^{CTF18}. Error bars, SEM; n > 500 for each experiment.

clones known to be enhanced in the *rrf-3* RNAi-sensitive background. We observed no enhancement of the RNAi phenotype in the *him-1* mutant compared with wild type in all 17 cases (data not shown) and therefore conclude that the increases in Pvl are due to bona fide genetic interactions.

Colon tumor sequencing identified multiple mutations in cohesin components, highlighting cohesin as a major mutational target. We predicted that the interactions we observed with *him-1/SMC1* would also be observed with mutations in the other cohesion subunits. Two additional *C. elegans* mutant strains defective for cohesin components were analyzed: *smc-3(ok1703)* and *scc-1(ok1017),* both of which are null mutants. Ninety percent of *smc-3* mutant homozygotes reach adulthood and are sterile. The remaining 10% of progeny arrest at the L3 or L4 larval stage, and 8.6 ± 2% of the sterile adults exhibited the Pvl phenotype. *scc-1* mutant homozygotes had a similar phenotype to *smc-3*; 50% of larva arrest, the remaining 50% that reach the adult stage are sterile, and 10.3 ± 2% of these adult worms are Pvl. Although these mutations are knockouts of essential genes, we were able to test for genetic interactions using the Pvl assay because those animals reaching the adult stage often developed a wild-type vulva, probably due to maternal contribution of wild-type protein. The fact that null mutations and knockdown of essential genes can be assayed with this system is a major advantage over other multicellular genetic screening systems. Both *smc-3(ok1703)* and *scc-1(ok1017)* mutants showed synthetic interactions with *crn-1/RAD27*, *F17C11.10/CTF4*, *Y47G6A.8/CTF18*, *T22C1.4/CTF8*, and *K09H9.2/DCC1* in the RNAi assay (Figure 5, B and C). We conclude that these subunits of the multiprotein cohesin complex share at least a subset of genetic interactions in both yeast and worms and this further emphasizes their importance as potential therapeutic targets.

Genetic Interactions Are Specific

To determine if the observed Pvl enhancement was a general effect caused by RNAi treatment, we tested *him-1(e879)* with 15 different control RNAi clones, 10 of which were randomly chosen from chromosome I. None of the 15 RNAi experiments resulted in a significant increase in the frequency of Pvl with either N2 or *him-1(e879)* (Figure 6A). This demonstrated that the increase in Pvl frequency observed when the cohesin mutants are treated with RNAi against *crn-1/RAD27*, *F17C11.10/CTF4*, *Y47G6A.8/CTF18*, *T22C1.4/CTF8*, and *K09H9.2/DCC1* are specific. Furthermore, three of the control RNAi clones targeted DNA metabolism/repair genes, *xpa-1/XPA, rad-51/RAD51,* and *cku-70/CKU70.* Notably, *him-1(e879)* did not show an interaction when treated with these DNA repair gene targeting RNAi clones, thereby demonstrating that the interaction is not due to a general interaction with DNA metabolism genes. Additionally, we tested whether *him-1(e879)* interacted with *fkh-10* and *F31C3.2,* two genes that were previously shown to interact with the checkpoint gene *mdf-1/MAD1* in *C. elegans* (Tarailo *et al.,* 2007). We did not observe a synthetic increase in Pvl frequency after treatment with the control RNAi constructs, indicating that the interactions identified for the cohesins are specific.

To test whether *RAD27*, *CTF4,* and members of the alternative RFC^{CTF18} were specifically interacting with the cohesion mutants or with chromosome instability mutants in general, we tested another *C. elegans* chromosome instability mutant, *him-6(ok416). him-6* is the RecQ helicase most similar to *BLM* in humans, which is mutated in a variety of tumors (Wicky *et al.,* 2004; Grabowski *et al.,* 2005; Ouyang *et al.,* 2008). *him-6* homozygotes are viable and exhibit chromosome nondisjunction that results in a high incidence of males and dead aneuploid embryos. *him-6(ok416)* have a low frequency of Pvl (5.9 ± 0.5%). A statistically significant increase in Pvl frequency was observed in *him-6*; *crn-1/RAD27(RNAi)* and *him-6; F17C11.10/CTF4(RNAi)* animals but not in *him-6* mutants treated with RNAi against components of the alternative RFC^{CTF18} (Figure 6B). This demonstrated that different CIN mutants have specific interaction profiles.

DISCUSSION

In this work we have identified a set of genetic interactions that are conserved between *S. cerevisiae* and *C. elegans*. *RAD27, CTF4, CTF8, CTF18,* and *DCC1* are potential therapeutic targets because they each interact with multiple colon cancer mutated genes, giving them the potential to selectively eliminate cancerous cells with a variety of mutational backgrounds.

We have found that at least a subset of genetic interactions are common for members of the same protein complex. Null mutations of *smc-3* and *scc-1* in *C. elegans*, two additional components of cohesin, share the same tested interactions as *SMC1/him-1.* The fact that members of the same protein complex share genetic interactions is reasonable from a functional standpoint and has been previously observed and used to identify new protein complexes or new members of known complexes (Collins *et al.,* 2007). Additional work in this area has the potential to identify additional cohesin components and to further elucidate the individual roles of known components.

We have also found that *him-6*, the *C. elegans* ortholog of the human *BLM* gene, genetically interacts with *CTF4* and *RAD27* but not with components of the alternative RFC. This finding suggests that therapeutic targeting of either *CTF4* or *RAD27* may also be effective in the treatment of cancers

harboring mutations in *BLM*, such as certain leukemias. Our results collectively demonstrate that different CIN mutants have specific interaction profiles. This in turn suggests that different types of cancer will respond uniquely to chemotherapeutic strategies and that this difference will be in part dependent upon a cancer's specific mutational profile. For this reason it is important to expand our current genetic networks both in yeast and in more complex multicellular animals that have a greater gene repertoire and a genome more similar in complexity to a human genome. For the purpose of cancer therapeutic target identification, focus should be placed on mapping the genetic interactions of the genes most often mutated in a wide variety of tumor types.

The experimental approach presented in this work is well poised to comprehensively elucidate the genetic interactions, both essential and nonessential, of genes known to be mutated in tumors. Interestingly, mutagenesis screens using the Pvl phenotype as a readout have been performed previously; however, the goal of these screens was to identify genes involved in vulval development (Seydoux *et al.*, 1993; Eisenmann and Kim, 2000). These types of screens identified a host of genes involved in diverse signaling pathways such as EGF, RAS, NOTCH, and WNT signaling, all of which are required for correct vulval development. Mutants in vulval signaling and/or development result in a range of phenotypes including egg-laying defective (Egl), vulvaless (Vul), and Pvl. Additional mutants with a role in cell division were later recovered from these banks of Pvl mutants, including *evl-14/PDS5* and *scc-3/SCC3* (Wang *et al.*, 2003). This latter finding demonstrated that the Pvl phenotype could also be used to identify cell division mutants. Additionally, several genes known to be involved in the cell cycle, such as *air-1/aurora kinase*, *air-2*, *sep-1/separase*, *cdk-4/CDK4/6*, and *san-1/MAD3*, exhibit a Pvl phenotype when mutated (Park and Krause, 1999; Woollard and Hodgkin, 1999; Furuta *et al.*, 2002; Simmer *et al.*, 2003). Similarly, chromosome stability mutants, such as the catalytic subunit of telomerase, *trt-1*, are also Pvl (Meier *et al.*, 2006). These mutant phenotypes are consistent with the view that mutations in genes required for the cell division cycle or to maintain genomic integrity result in defects in the vulval postembryonic lineage and can give rise to the Pvl phenotype.

In theory, defects in any postembryonic lineage could be used to identify cell division cycle mutants. O'Connell *et al.* (1998) used this fact to screen for temperature-sensitive cell division mutants by screening for animals with the Stu (sterile and uncoordinated) phenotype. We reasoned that defects in the postembryonic lineages could be exploited to identify combinations of mutants that result in cell proliferation defects, and we chose to use the vulval lineage for genetic screening because defects are easily visually identified and quantifiable. Some of the advantages of this technique result from the single generation screening approach that has allowed us to interrogate the interactions of essential genes.

Our work sheds some light on the question of whether or not genetic interaction networks are conserved between organisms. Previous studies by Lehner *et al.* (2006) and Byrne *et al.* (2007) focused on genes involved in signaling pathways and suggested that a relatively low percentage of genetic interactions are conserved. On the other hand, work by Tarailo *et al.* (2007) that focused on the spindle assembly checkpoint suggested that a much higher fraction of interactions, on the order of 50%, are conserved between *S. cerevisiae* and *C. elegans*. Recent work by McManus *et al.* (2009) demonstrated the usefulness of a cross-species candidate approach for drug target identification. They showed that an interaction first identified in yeast between *RAD27* and *RAD54* (Symington, 1998) was conserved in HCT116 cells.

Our data suggest that there is a high degree of conservation between species, at least with respect to essential genes involved in chromosome biology. One caveat of our cross-species candidate approach is that the selection pressures that shape genetic networks in a tumor may be quite different from those in an organism. Further genetic interaction testing of essential genes in model organisms, tumor cell lines, and ultimately patient tumor samples, will be required to address this issue.

The interaction network in yeast and the conserved interactions identified in the somatic cell divisions of *C. elegans* point to *CTF4*, *RAD27*, and members of the alternative RFC^{CTF18} as important therapeutic targets worthy of further investigation. The alternative RFC components are especially attractive as therapeutic targets because of their relative lack of phenotype when knocked down singly in *C. elegans* and mammalian cells. On the other hand, others have found that single knockdown of *CTF4* or *RAD27* has a detrimental effect on otherwise normal cells (Zhu *et al.*, 2007, Saharia *et al.*, 2008). The ideal cancer therapeutic would eliminate cancerous cells while causing no harm to normal cells. Potentially, many genes have a SL relationship with genes mutated in cancer, but the best therapeutic targets are those that cause the least amount of harm to normal body cells during treatment. For genes whose knockdown causes a detrimental effect on normal cells it may be a matter of titrating the therapeutic dose to induce selective killing of cancerous cells.

The approach presented in this article identified *DCC1*, *CTF8*, and *CTF18* as ideal potential cancer therapeutic targets. The *C. elegans* Pvl assay is a powerful tool for many reasons. It is a convenient approach to test for conservation of genetic interactions that cause synthetic lethality and were identified in simpler organisms. This will help to prioritize testing of interactions in mammalian cells with the ultimate goal of therapeutic target identification and drug development. The Pvl assay also offers the possibility of screening for new SL genetic interactions not present in yeast. This type of testing in a multicellular animal, with a genome similar in complexity to a human genome, may greatly benefit our understanding of genes that cause slow growth or lethality in combination with genes mutated in cancers, thereby expanding our list of potential therapeutic targets.

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