Chromosomal Instability Confers Intrinsic Multidrug Resistance

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Abstract

Aneuploidy is associated with poor prognosis in solid tumors. Spontaneous chromosome missegregation events in aneuploid cells promote chromosomal instability (CIN) that may contribute to the acquisition of multidrug resistance in vitro and heighten risk for tumor relapse in animal models. Identification of distinct therapeutic agents that target tumor karyotypic complexity has important clinical implications. To identify distinct therapeutic approaches to specifically limit the growth of CIN tumors, we focused on a panel of colorectal cancer (CRC) cell lines, previously classified as either chromosomally unstable (CIN+), and treated them individually with a library of kinase inhibitors targeting components of signal transduction, cell cycle, and transmembrane receptor signaling pathways. CIN+ cell lines displayed significant intrinsic multidrug resistance compared with CIN− cancer cell lines, and this seemed to be independent of somatic mutation status and proliferation rate. Confirming the association of CIN rather than ploidy status with multidrug resistance, tetraploid isogenic cells that had arisen from diploid cell lines displayed lower drug sensitivity than their diploid parental cells only with increasing chromosomal heterogeneity and isogenic cell line models of CIN+ displayed multidrug resistance relative to their CIN− parental cancer cell line derivatives. In a meta-analysis of CRC outcome following cytotoxic treatment, CIN+ predicted worse progression-free or disease-free survival relative to patients with CIN− disease. Our results suggest that stratifying tumor responses according to CIN status should be considered within the context of clinical trials to minimize the confounding effects of tumor CIN status on drug sensitivity. Cancer Res; 71(5); 1858–70. ©2011 AACR.

Introduction

Colorectal cancer (CRC) is associated with at least 2 distinct patterns of genomic instability (1). The more common form of genomic instability in CRC is chromosomal instability (CIN), resulting in ongoing numerical and structural chromosomal aberrations in cancer cells, leading to intratumoral heterogeneity (2, 3). The less common pattern is microsatellite instability (MIN) caused by a deficiency in the mismatch repair apparatus. Because of the accumulation of replication errors, MIN results in length variation of microsatellite sequences in DNA. Most MIN+ CRC cell lines are near-diploid (4, 5) and chromosomally stable (CIN−). In contrast, CIN+ CRC cell lines are aneuploid and display a higher frequency of chromosomal missegregation errors during each mitosis relative to diploid cells (2). In human CRC, CIN+ is widely inferred through the measurement of tumor DNA ploidy (6); normal diploid cells are defined with a DNA index of 1.0 (7) and thus an increase in DNA index infers polyploidy or aneuploidy. Approximately 25% of human CRC are both CIN− and MIN− (6).

Consistent molecular mechanisms responsible for the CIN+ phenotype and hence means to target this pattern of genome instability in colorectal and other solid tumors remain poorly defined. Putative mechanisms that may contribute to CIN include weakening of the spindle assembly checkpoint (SAC; refs. 8, 9), defective sister chromatid cohesion (10), merotelic sister chromatid attachments (11), defective cytokinesis (12), centrosome amplification (13), and chromosome breakage–fusion–bridge cycles (14).
Increasing evidence suggests that CIN is associated with poor prognosis in solid tumors (6, 15, 16). It has been suggested that adverse outcome associated with CIN may be related to increased tumor cell heterogeneity, driving the ability of tumors to adapt to environmental stresses (17–19). Consistent with a hypothesis whereby CIN may enhance tumor adaptation, transient initiation of CIN, following the brief induction of MAD2 expression in activated KRAS-initiated mouse lung tumor models, is associated with a high frequency of tumor recurrence following withdrawal of the KRAS oncogenic stimulus (20). Preclinical studies have shown that CIN is associated with the rapid acquisition of multidrug resistance in cell line systems (21) and intrinsic taxane resistance in vitro and in vivo (22). Recently, we and others have proposed the existence of a CIN survival phenotype that allows CIN+ tumor cells to tolerate the impact of excessive chromosome gains and losses (22–24) that may in turn impact upon altered drug sensitivity.

Determining how CIN might impact upon prognosis and how this pattern of genomic instability might be specifically targeted remains an important research area (23, 25). Evidence in lower eukaryotes has shown that aneuploid Saccharomyces cerevisiae are dependent on increased glucose utilization and are more sensitive to both heat shock protein 90 and proteosome inhibitors (26). Polyploid S. cerevisiae are dependent upon increased expression of genes involved in sister chromatid cohesion and mitotic spindle function (27). Rorschke and Kirsch have shown the existence of anticancer compounds that may specifically target karyotypically complex cancer cells (25). These observations indicate that karyotypic instability may be specifically targeted in eukaryotic organisms and suggest that CIN might be an exploitable and targetable phenotype in cancer.

To identify distinct therapeutic approaches to limit the growth of CIN+ tumors relative to diploid cells, we focused on a panel of CRC cell lines that had previously been classified as CIN+ or CIN− and used kinase inhibitor and cytotoxic libraries to identify agents that might be preferentially lethal toward CIN+ cells. Both isogenic and nonisogenic CIN+ cell lines displayed intrinsic multidrug resistance in vitro relative to CIN− cell lines. Importantly, consistent with the proposal that CIN+ is associated with intrinsic multidrug resistance, in a meta-analysis of patient outcome in CRC, CIN+ was associated with significantly worse clinical outcome relative to diploid cancers in both early- and late-stage disease following cytotoxic therapy.

Materials and Methods

Cell lines and FISH analysis
27 CRC cell lines (Table 1; Supplementary Table 1), previously characterized for numerical/structural CIN, MIN status (2, 28–30), and subject to Affymetrix SNP 6.0 Array analysis where available (20/27 cell lines; Wellcome Trust Sanger Institute), were used. We used publicly available somatic mutation data from the Sanger Institute Cancer Cell Line Project (CLP) and COSMIC database (31). Fifteen CIN+ and 6 CIN− cell lines used in our analysis were present within the CLP database, and a total of 20 of the 61 genes resequenced in the project were found to have somatic mutations in at least 1 of those 21 cell lines. Additional information regarding the somatic mutation status of APC, CTNNB1, KRAS, MLH1, PIK3CA, and TP53 were obtained from both published (32–35) and internal laboratory data. Isogenic HCT116 MAD2+/- cell lines (9) and HCT116 PTG1+/- cell lines (10) were donated courtesy of Drs. Benezra and Vogelstein, respectively. To generate tetraploid HCT116 cells, naturally occurring tetraploid cells were isolated from the parental cell line and single cell sorted using flow cytometry. Clonal FISH was performed with centromere enumeration probes against centromeres on chromosomes 2 and 15.

Calbiochem Kinase Inhibitor Library and 5-FU screen
Calbiochem Kinase Inhibitor Libraries I and II (EMD Biosciences) containing 160 inhibitors were used. Comprehensive data for these inhibitors including references documenting target inhibition or downstream signaling cascade inactivation can be found on the manufacturer’s Web site (36). Cells were plated into 96-well tissue culture microplates at an initial seeding density of 4,000 cells per well. After 24 hours, cells were treated with the inhibitors at a final concentration of 10 μmol/L per well. This concentration was selected following drug titration test analysis to give an optimal range of mean relative surviving cells following inhibitor treatment across all cell lines. After 72 hours of treatment, cell viability was assayed using the Celltiter-Blue Cell Viability Assay (Promega) according to the manufacturer’s instructions. The fluorescent readout for each inhibitor treated well was normalized to vehicle control wells. For the HCT116 MAD2 isogenic cell lines, a final inhibitor concentration of 1 μmol/L per well was used to allow for a larger range of surviving cells across all inhibitors.

5-Fluorouracil (5-FU) was used at both 1 and 10 μmol/L for a treatment length of 72 hours. The 27 CRC cell lines were plated and assayed using the same conditions and methods as described earlier.

Biolog Anti-Cancer Agent Microplate screen
Biolog Anti-Cancer Agent Microplates M11–M14 consisting of 92 anticancer agents at 4 increasing concentrations between 0.1 and 25 μmol/L per agent were used. Cells were plated at an initial seeding density of 5,000 cells per well. After 72 hours, the number of surviving cells per well was assayed using Biolog Redox Dye Mix MA according to manufacturer’s instructions and normalized to the negative control wells.

Cell proliferation assay
We measured the proliferation rate of all 9 CIN− and 18 CIN+ CRC cell lines, using the IncuCyte Long-term Cell Imaging System. Cell lines were plated in 96-well plates at an initial plating density of 4,000 cells per well and phase-contrast images were obtained every 2 hours over 70 hours, allowing measurements of cell monolayer confluence. Outliers were removed manually, and growth curves were fitted by splines with the R package smooth (37) with smoothing parameter smooth.gcf = 0.7.
Table 1. Cell lines used in this study

<table>
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<th>Cell Line</th>
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<th>CIN status</th>
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NOTE: Black indicates presence of mutation; white indicates absence of mutation; and gray indicates mutation status not known.
Meta-analysis of clinical studies
Survival data were summarized using a log hazard ratio for comparison between CIN\(^+\) and CIN\(^-\) groups. Data from individual studies were extracted using the methods described by Parmar and colleagues (38) and pooled to generate the summary statistic and CIs using a fixed-effects model with inverse variance weighting. All meta-analyses were performed using Stata 10.1 (StataCorp).

Statistical methods
All tests were performed as 2-sided unless otherwise mentioned. To remove outliers, drugs resulting in relative number of cells greater than 1.4 were eliminated from analysis. A Kolmogorov–Smirnov test was performed to test for overall differences in the distribution of relative cell numbers following inhibitor treatment between CIN\(^+\) and CIN\(^-\) cell lines. Inhibitors that showed a fraction of surviving cells greater than 0.8 in more than 75% of the cell lines were excluded from the analysis. For comparisons of 2 cell lines, drugs resulting in relative number of cells greater than 0.8 in both cell lines were removed from analysis. A Wilcoxon signed-rank test was used to test for differences between CIN\(^+\) and CIN\(^-\) cells (for each concentration of drug in Biolog microplates). For the Biolog microplates, each concentration of drug was used in duplicate; therefore, the replicates which showed the least difference in cell number between the 2 cell lines was used for further analysis.

The maximal slope of the growth curve (μ) for each cell line was used to test whether the difference in drug sensitivity between CIN\(^+\) and CIN\(^-\) colorectal cancer cell lines was not solely due to different proliferation rates. CIN\(^+\) and CIN\(^-\) cell lines with μ < 1 were tested with a one-sided Wilcoxon–Mann–Whitney test. Next, we corrected for the influence of the proliferation rates of each cell line. We estimated a linear regression model for all cell lines with the mean fraction of surviving cells over all inhibitors as a dependent variable, μ as a linear, independent variable, and CIN status as a factor variable. The interaction term was utilized to test for significant differences in slopes between CIN\(^+\) and CIN\(^-\) cell lines.

To test for significant differences in sensitivity to thymidylate synthase inhibitors comparing HCT116 diploid parental and PTTG1\(^+/–\) or MAD2\(^+/–\) cells, we corrected for the influence of different concentrations by estimating a linear regression model for near linear correlations between concentration and sensitivity or a one-way ANOVA otherwise. In each case, the concentration was used as the independent variable and the resulting residuals were tested for differences between CIN\(^+\) and CIN\(^-\) cells with a Wilcoxon–Mann–Whitney test.

All statistical analyses were performed in R and can be found in the Supplementary Sweave document.

Results

Classification of CIN\(^+\) cell lines and relationship with ploidy status and structural chromosomal complexity
We selected 9 CIN\(^-\) and 18 CIN\(^+\) CRC cell lines (Table 1). CIN status for these cell lines had been previously described in terms of numerical and structural chromosomal aberrations (2, 28–30).

To confirm the utility of published approaches for defining the CIN status of the cell lines, where SNP Array data were available, we estimated the ploidy status of the cell lines by using weighted mean integer copy numbers derived from the PICNIC (Predicting Integral Copy Numbers In Cancer) algorithm (ref. 40; Fig. 1A). Ploidy estimates classified cells as CIN\(^+\) if they surpassed a threshold of ploidy greater than 2.2 (corresponding to an estimated DNA index of 1.1). In addition, modal chromosomal number as determined previously using high-quality metaphase spreads (28, 29) correlated well with ploidy estimates derived from weighted mean PICNIC copy number analysis using the SNP Array data [Pearson’s correlation coefficient (CC) = 0.94, Pearson’s correlation test \(P < 0.0001\); Fig. 1B]. These data support the utility of SNP Array data for estimating cell line ploidy status and are consistent with ploidy estimates by traditional measures, confirming the CIN status of cell lines used in this analysis.

Next, we addressed the relationship between CIN status and structural chromosomal complexity, using a summary structural chromosomal complexity score (SCCS) derived from the SNP Array data sets. This SCCS was determined by summarizing (i) the number of break points, (ii) LOH events as predicted using PICNIC (40), and (iii) the Genome Integrity Index (GII; ref. 41) into a single value for each cell line (Fig. 1C). There was a highly significant correlation between ploidy status and the SCCS (Pearson’s CC = 0.746, \(P = 0.0002\)). Taken together, these analyses confirm that cells classified as CIN\(^+\) have significantly greater ploidy and structural chromosomal complexity than CIN\(^-\) cells.

CIN\(^+\) status is associated with intrinsic multidrug resistance
Next, we aimed to determine whether a specific kinase inhibitor could be identified to selectively target CIN\(^+\) cell lines. We used a small molecule library (Calbiochem Kinase Inhibitor Library I and II) that included 160 inhibitors to treat the 18 CIN\(^+\) and 9 CIN\(^-\) cell lines. Preliminary drug titration experiments revealed that the majority of the CIN\(^+\) cell lines were resistant to concentrations up to 1 \(\mu\)mol/L (Supplementary Fig. 1) and therefore 10 \(\mu\)mol/L was selected as the optimal drug concentration for cell growth inhibition across the majority of cell lines in order to attempt to identify drugs that were specifically active in CIN\(^+\) cells. No specific inhibitor or inhibitor family was found to be preferentially active in CIN\(^+\) cell lines compared with CIN\(^-\) cell lines. In contrast, CIN\(^+\) cancer cell lines were significantly more resistant to the inhibitors (Kolmogorov–Smirnov test, \(P < 0.0001\); Fig. 2A–C). Following correction for multiple testing hypotheses, 45 inhibitors were identified which showed significantly greater...
activity in CIN− cell lines than in CIN+ cell lines (Supplementary Table 2).

To address whether the variation in drug sensitivity between the CIN− and CIN+ cell lines was attributable to differences in proliferation rate, we calculated the maximum growth rate (μ) for each cell line. We observed that CIN− and CIN+ cell lines show significantly different slopes in regression lines (t test, \( P = 0.007 \)), with CIN+ cell lines showing a higher proliferation rate than in the CIN− cell lines (Wilcoxon–Mann–Whitney test, \( P = 0.003 \)). These data are consistent with previous studies, suggesting that aneuploidy (42, 43) or chromosomal segregation defects (11, 44) have a negative impact on cellular proliferation rate. We observed a significant correlation between increased sensitivity to the inhibitors at higher proliferation rates for CIN+ cell lines (Pearson's CC = 0.61, \( P = 0.007 \)). In contrast, no such correlation was observed between proliferation rate and drug sensitivity in CIN− cell lines (Pearson's CC = 0.24, \( P = 0.55 \); Supplementary Fig. 2A).

Neither ploidy index nor the SCCS showed a significant correlation between increased sensitivity to the inhibitors at higher proliferation rates for CIN+ cell lines (Pearson's CC = 0.94, \( P < 0.0001 \)). In contrast, no such correlation was observed between proliferation rate and drug sensitivity in CIN− cell lines (Pearson's CC = 0.24, \( P = 0.55 \); Supplementary Fig. 2A). Neither ploidy index nor the SCCS showed a significant correlation with proliferation rate (data not shown).

We next investigated whether CIN+ and CIN− cell lines with similar proliferation rates displayed differential drug sensitivity (cell lines with \( \mu < 1 \); 22/27 cell lines). CIN+ cell lines remained multidrug resistant compared with CIN− cell lines within this group (one-sided Wilcoxon–Mann–Whitney test, \( P = 0.013 \)). Next, we used a more conservative approach and corrected for the influence of proliferation rate of each individual cell line within this group. CIN+ cell lines remained significantly more drug resistant than CIN− cell lines (one-sided Wilcoxon–Mann–Whitney test, \( P = 0.049 \); Supplementary Fig. 2B). These data suggest that at similar growth rates, CIN+ cell lines remain more drug resistant than CIN− cell lines, indicating that proliferation rate is unlikely to be the main determinant of drug sensitivity.

**Somatic mutation status and drug sensitivity**

Next, we addressed whether distinct tumor cell line somatic mutations might be the underlying determinant of drug resistance rather than genomic instability status. We investigated whether the somatic mutation status of 20 genes (Table 1) was associated with altered sensitivity either to inhibitors grouped according to target kinase family (Aurora kinase, AKT, CDK, EGFR, FLT-3, GSK-3, JAK3, JNK, MEK, PDGFR, PI3K, and SYK) or to all inhibitors combined. We addressed whether there was an association of drug sensitivity with somatic mutation status in 13 genes for which cell line group sizes had sufficient statistical power to compare drug sensitivity in wild-type compared with mutated cell lines.

![Figure 1](cancerres.aacrjournals.org)
PI3CA mutation was the only somatic mutation significantly associated with altered sensitivity to inhibitors grouped according to target kinase families [PI3CA mutation associated with increased sensitivity to inhibitors targeting AKT, Aurora kinase, EGFR, PDGFR, and PI3K, Wilcoxon–Mann–Whitney test, corrected $P = 0.003$.]
PIK3CA and drug sensitivity (data not shown). Notably, Supplementary Fig. 3B. We found no evidence for a specific models. These data suggest that CIN+PTTG1 or independent (45), CIN assumption that the tests are either positively correlated (Fig. 3C and D). explain the altered drug sensitivity in the CIN+ containing 92 anticancer cytotoxic agents. Consistent with the diploid HCT116 cell lines with the Biolog cytotoxic library treated the isogenic HCT116 numerical CIN relative to its diploid parental cell line (9). We loss of 2 distinct proteins controlling mitotic fidelity, is by ongoing chromosome missegregation events driven by surviving cell line fraction after treatment. Consistent with the diploid cell line with the kinase inhibitors and assessed the isogenic CIN+ CRC cell lines display intrinsic multidrug resistance To further test the association of CIN with intrinsic drug resistance to drugs other than kinase inhibitors, we challenged CRC cell lines display intrinsic multidrug resistance. Consistent with this hypothesis, both isogenic MIN, in conferring altered drug sensitivity.

**Isogenic CIN+ CRC cell lines display intrinsic multidrug resistance**

To support a direct role for the contribution of CIN to the multidrug-resistant phenotype, we assessed whether drug sensitivity was altered in isogenic CRC models of CIN. The HCT116 MAD2+/− cell line has one allele of the SAC gene, MAD2, deleted by homologous recombination, resulting in numerical CIN relative to its diploid parental cell line (9). We treated the isogenic HCT116 MAD2+/− cell and its parental diploid cell line with the kinase inhibitors and assessed the surviving cell line fraction after treatment. Consistent with the nonisogenic cell line data presented previously, the MAD2+/− cell line was found to be more resistant overall to the inhibitors tested than to the parental diploid cell line (one-sided Wilcoxon signed-rank test, P = 0.001; Fig. 3A and B). No single inhibitor seemed to specifically target the HCT116 MAD2+/− cell line.

To further test the association of CIN with intrinsic drug resistance to drugs other than kinase inhibitors, we challenged the HCT116 MAD2+/− and another CIN+ isogenic cell line, HCT116 PTTG1−/−, together with their isogenic parental diploid HCT116 cell lines with the Biolog cytotoxic library containing 92 anticancer cytotoxic agents. Consistent with the data from the kinase inhibitors, both the CIN+ MAD2+/− and PTTG1−/− cells were significantly more resistant (one-sided Wilcoxon signed-rank test, P < 0.001, except P = 0.035 at the lowest concentration of drug for PTTG1−/−) to a diverse range of anticancer agents than to the parental diploid cell lines (Fig. 3C and D).

Importantly, both the HCT116 MAD2+/− and parental diploid cell lines continue to display MIN+ (Supplementary Fig. 4), indicating that MIN status is unlikely to sufficiently explain the altered drug sensitivity in the CIN+ isogenic models. These data suggest that CIN+ status, initiated by ongoing chromosome missegregation events driven by loss of 2 distinct proteins controlling mitotic fidelity, is the dominant phenotype associated with altered drug sensitivity.

**CIN+ not tetraploidy is associated with drug resistance**

CIN+ CRC cell lines missegregate chromosomes at a high rate, in contrast to CIN− CRC cells that have a lower frequency of mitotic errors (1, 2). In addition, CIN− cells fail to tolerate the propagation of CIN when chromosome segregation errors are artificially induced by drug treatment (11), suggesting that sustaining CIN in a cell population may require a specialized survival phenotype. The majority of CIN+ CRC cell lines used in this study are triploid or tetraploid. We therefore considered whether altered ploidy status, rather than ongoing CIN, might be associated with enhanced drug resistance. Clonal tetraploid and diploid HCT116 cells were treated with the kinase inhibitors. There was no significant difference in the relative number of surviving cells following drug treatment between HCT116 tetraploid clone 4 (TC4) cell line and diploid clone 8 (DC8) cell line. However, tetraploid clone 9 (TC9) was significantly more resistant than the DC8 cell line (Wilcoxon signed-rank test, P < 0.001; Fig. 4A). Further investigation by clonal FISH (Fig. 4B) revealed that TC9 had a more heterogeneous karyotype compared with TC4 cell lines, with a significantly higher proportion of cells that deviated from the mode of 4 copies of both chromosomes 2 and 15 (Fisher’s exact test, P = 0.05; Fig. 4C). This implies that karyotypic heterogeneity, rather than increased ploidy, might be responsible for increased drug resistance compared with karyotypically stable diploid cells. We cannot formally exclude the possibility that acquired mutations present in the drug-resistant tetraploid clone that may have permitted the spontaneous tetraploid phenotype may primarily be responsible for increased drug resistance.

Taken together with the isogenic cell line data sets presented here, where CIN is artificially induced through loss of one allele of MAD2 or both copies of PTTG1, these results support the contribution of CIN, rather than increased ploidy status or MIN, in conferring altered drug sensitivity.

**Relationship between CIN status and benefit from cytotoxic therapy in clinical datasets**

Published clinical data support the view that CIN+ CRC is associated with a worse prognosis compared with CIN− tumors (6), and data presented here suggest that CIN+ cell lines display intrinsic multidrug resistance. Conceivably, the poorer prognosis of CIN+ disease may relate in part to intrinsic drug resistance of thymidylate synthase inhibitors, cytotoxics commonly used in the adjuvant treatment of CRC. Consistent with this hypothesis, both isogenic CIN+ cells are significantly more resistant to the majority of thymidylate synthase inhibitors tested, including 5-FU, and non-isogenic CIN+ cells are more resistant to 5-FU than to CIN− cell lines, at physiologic concentrations (Supplementary Fig. 6A, B).

Next, we asked whether CIN+ status might be associated with poorer outcome following adjuvant therapy with 5-FU-based regimens. A meta-analysis of studies examining the relationship between CIN and prognosis in locoregional CRC, revealed that CIN+ disease (defined as aneuploidy/polyplody determined using flow cytometry) confers a worse overall survival (30 studies) (Supplementary Fig. 5A) and
Figure 3. A, boxplot showing that following treatment with kinase inhibitors, there seemed to be a higher surviving fraction of cells in the HCT116 MAD2+/+ cell line than in its parental diploid cell line (P < 0.001) following treatment with each equivalent inhibitor. B, heatmap showing the relative numbers of surviving cells following the inhibitor treatments compared with vehicle control across the HCT116 MAD2+/+ and parental diploid cell lines (inhibitors that show a surviving cell fraction of >0.8 in both cell lines have been excluded). C, Biolog M11–M14 drug microplates were used at 4 increasing concentrations per drug (0.1–25 μmol/L) to treat HCT116 MAD2+/+ and PTTG1−/− and their parental diploid cell lines for 72 hours. The boxplot shows difference in relative surviving cell number across all drugs at each of the 4 concentrations, comparing MAD2+/+ and PTTG1−/− cells to their specific isogenic parental cells. Significant P values suggest higher resistance in MAD2+/+ or PTTG1−/− cells than in their parental diploid cells. D, heatmap of surviving fraction of cells compared with negative control in HCT116 MAD2+/+, PTTG1−/−, and their parental diploid cell lines treated with Biolog drug microplates at 4 increasing concentrations per drug (1–4, 1 being the lowest). Drugs resulting in a surviving cellular fraction of more than 0.8 compared with negative control in both isogenic cell lines were excluded.
Figure 4. A, boxplot of relative surviving cell numbers comparing HCT116 TC4 and DC8 cell lines and TC9 with DC8 cell lines. The TC9 cell line was significantly more resistant than DC8 ($P < 0.001$). The difference in drug sensitivity between TC4 and DC8 was not significant ($P = 0.078$). B, representative FISH images for TC4 and TC9. Probes against chromosome 15 in green. C, histogram showing distribution of number of markers per cell corresponding to chromosome 2 (top two) and 15 (bottom two) in TC4 and TC9. TC9 had a statistically significant higher proportion of cells that deviated from having 4 copies of both chromosomes 2 and 15 ($P = 0.05$) than that in TC4.
progression-free survival (15 studies) (Supplementary Fig. 5B) compared with patients with diploid CRC. Similarly, if only patients who received chemotherapy are included (2 studies; ref. 46, 47), CIN$^+$ tumors are associated with a worse overall survival (Fig. 5A). Two studies explore the predictive value of CIN (46, 48) in patients with locoregional CRC who received either adjuvant chemotherapy or no chemotherapy following surgery (Fig. 5B). Patients with diploid CRC seem to benefit more from chemotherapy than patients with CIN$^+$ tumors. Combined analysis of CIN$^+$ and diploid patients shows similar magnitude of benefit as would be expected from literature (bottom; ref. 49).

Figure 5. A, impact of CIN$^+$ on disease-free survival, overall survival, and those receiving adjuvant chemotherapy in locoregional CRC. Overall CIN$^+$ seems to confer a worse prognosis compared with diploid. B, benefit derived from adjuvant 5-FU in patients with (near) diploid (top) and CIN$^+$ (middle) CRC. Patients with diploid CRC seem to benefit more from chemotherapy than patients with CIN$^+$ tumors. Combined analysis of CIN$^+$ and diploid patients shows similar magnitude of benefit as would be expected from literature (bottom; ref. 49).

progression-free survival (15 studies) (Supplementary Fig. 5B) compared with patients with diploid CRC. Similarly, if only patients who received chemotherapy are included (2 studies; ref. 46, 47), CIN$^+$ tumors are associated with a worse overall survival (Fig. 5A).

Two studies explore the predictive value of CIN (46, 48) in patients with locoregional CRC who received either adjuvant chemotherapy or no chemotherapy following surgery (Fig. 5B). Patients with diploid CRC seem to benefit from 5-FU–based therapy ($N = 262$, HR = 0.61; 95% CI = 0.40–0.94, $P = 0.024$; $I^2 = 0\%$, $P = 0.467$) compared with untreated diploid controls, whereas there was no significant difference between treated and untreated CIN$^+$ CRCs ($N = 303$, HR = 0.81; 95% CI = 0.57–1.16, $P = 0.250$; $I^2 = 0\%$, $P = 0.932$). The combined analysis of all patients suggests a benefit following 5-FU treatment comparable with that reported in the literature for genetically unselected patients (49). While these studies are limited, they are consistent with the view that patients with CIN$^+$ CRC derive less benefit from 5-FU–based adjuvant cytotoxic chemotherapy than patients with diploid CRC. Prospective evaluation of the association of CIN$^+$ disease with the efficacy of combination regimens (e.g., 5-FU/oxaliplatin) in stage 3 and 5-FU/oxaliplatin and irinotecan in stage 4 disease might be considered to assess whether clinical outcome following these more recent regimens has a similar association with CIN status.
Discussion

In this analysis, we have provided evidence that CIN\(^{+}\) CRC cell lines display intrinsic multidrug resistance compared with CIN\(^{-}\) cell lines. No specific kinase inhibitor was identified that displayed greater activity in CIN\(^{+}\) cell lines. We cannot exclude the potential for off-target effects at the concentrations of kinase inhibitors used in this analysis; however, the same conditions were applied to the CIN\(^{-}\) cells and therefore off-target phenomena are unlikely to change the conclusions of this work. Furthermore, in the isogenic systems, we observed significant drug resistance in the CIN\(^{+}\) cell lines relative to their isogenic parental CIN\(^{-}\) pairs at all concentrations of cytotoxics tested (ranging from 0.1 to 25 \(\mu\)mol/L). Intriguingly, the isogenic CIN\(^{+}\) and tetraploid cell line systems suggest that the primary association is between multidrug resistance and CIN\(^{+}\) rather than tetraploidy. It has been previously shown that aneuploid cell lines can acquire multidrug resistance at an accelerated rate (50) that may be driven by cancer cell heterogeneity resulting from multiple chromosomal rearrangements in aneuploid cells. The short time course of our experiments in comparison with this study suggests that multidrug resistance is likely to be an intrinsic property of CIN\(^{+}\) cells rather than a process that is acquired in our cell systems over multiple generations. What might contribute to this intrinsic multidrug resistance phenotype in CIN\(^{-}\) cells? We speculate that either basal population heterogeneity in CIN\(^{+}\) cell lines is sufficiently diverse to confer a cell viability advantage following drug exposure or there is a specific CIN\(^{-}\) survival phenotype that initiates a tolerance of ongoing chromosomal rearrangements that is also associated with multidrug resistance.

There is increasing evidence in support of a CIN\(^{+}\) survival phenotype and putative molecular coordinators of this property. Cell death after mitotic arrest may result from transcriptional inhibition due to condensed chromatin, precipitating the degradation of short-lived mRNA encoding prosurvival proteins (51). CIN\(^{-}\) cells may overexpress these prosurvival genes compared with diploid cells (22) that may drive the resistance of CIN\(^{+}\) cells to a mitotic arrest triggered by taxanes. Jeganathan and colleagues have shown that tolerance of chromosome missegregation events can be conferred by a hypomorphic \(BUB1\) allele in mouse embryonic fibroblasts (24). Recently, Thompson and Compton have shown that chromosome missegregation in diploid human cells triggers an increase in nuclear \(p53\) and that \(p53\) null cells are able to tolerate chromosome missegregation events, enabling the propagation of aneuploid genomes (52). A higher proportion of the CIN\(^{+}\) cell lines used in our study have mutant \(p53\) in comparison with the CIN\(^{-}\) cell lines. However, when we pooled data for somatic mutation status and CIN status, CIN\(^{+}\) status was the only parameter significantly associated with resistance to these inhibitors.

Therefore, evidence exists for the coordination of apoptotic/cell death pathways following chromosome missegregation events. Conceivably, common molecular pathways regulating cell death following a chromosome missegregation event may become disrupted in CIN\(^{+}\) cells, simultaneously triggering tolerance of chromosome rearrangements and, as an indirect consequence, resistance to drug exposure.

The observations that CIN\(^{+}\) cancer cell lines seem to be less sensitive to a range of anticancer agents than to diploid cells and that poorer patient outcome follows cytotoxic treatment of CIN\(^{+}\) tumors compared with diploid counterparts strongly suggest the need to consider tumor stratification according to CIN status in the design of clinical trials testing novel anticancer agents in CRC. This is particularly relevant to the advanced CRC setting where the incidence of CIN\(^{+}\) is greater than in early-stage disease. Stratifying drug response according to CIN status may limit the risk of early drug attrition and heighten the chance of identifying responder populations in patients with diploid tumors. Importantly, these data indicate that specifically targeting cancer cells with CIN\(^{+}\) status, using currently available kinase inhibitors, seems challenging. An improved understanding of the mechanisms associated with the generation and survival of CIN\(^{+}\) CRC will be important to drive the development of new therapeutic approaches in order to improve patient outcome in this high-risk disease subtype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Chromosomal Instability Confers Intrinsic Multidrug Resistance

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