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GUCY2C reverse transcriptase PCR to stage pN0 colorectal cancer patients

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Abstract

The most important prognostic marker of survival and predictive marker of response to adjuvant chemotherapy in colon cancer patients is tumor cells in regional lymph nodes. Despite their importance, standard techniques to assess nodal metastases remain imperfect, as approximately 30% of patients with histology-negative lymph nodes (pN0) die of recurrent disease, reflecting occult metastases that escape detection. These observations highlight the clinical need for novel, accurate approaches to detect occult lymph node metastases in patients with colon cancer. GUCY2C is a biomarker whose expression normally is restricted to intestinal cells, but is near universally overexpressed by colorectal cancer cells. Recently, a prospective, multicenter, blinded clinical trial demonstrated for the first time that the prognostic utility of GUCY2C quantitative reverse transcriptase (qRT)-PCR to detect occult lymph node metastases in pN0 colorectal cancer patients. Molecular staging revealed that approximately 13% of pN0 patients were free of tumor cells, while approximately 87% had GUCY2C results that suggested occult metastases. The presence of occult lymph node metastases was the strongest independent predictor of time to recurrence and disease-free survival. These observations establish the utility of molecular detection of occult lymph node metastases for estimating prognostic risk in pN0 colorectal cancer patients. Advancing this molecular diagnostic into staging paradigms in clinical laboratories will require validation in independent patient populations, definition of the relationship between the quantity of occult tumor metastases and risk, and determination of the utility of GUCY2C qRT-PCR to identify pN0 patients who might benefit from adjuvant chemotherapy.

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Keywords

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Colorectal cancer is the fourth most common neoplasm, with approximately 150,000 new cases annually, and is the second leading cause of cancer-related mortality [1]. Colorectal cancer is responsible for approximately 10% of cancer-related deaths in the USA, with a mortality rate of approximately 50% [1–3]. Mortality reflects metastases: approximately 20% of patients have unresectable disease at presentation (stage IV) and more than 30% will develop metastases during the course of their disease [2–5]. Surgery continues to have the greatest impact on survival. However, while 'curative' surgery removes all detectable tumor and is most successful in early-stage disease, occult metastases result in relapse [1–3,6–9]. Recurrence rates range from approximately 10% for disease limited to mucosa (stage I) to greater than 60% for tumors metastatic to lymph nodes (stage III) [1–3,6–19].

Colorectal cancer staging

In colorectal cancer, the single most important prognostic determinant of survival is tumor cells in regional lymph nodes [1–6,9,20–24]. While histopathology remains the gold standard, staging imprecision by conventional microscopy reflects methodological limitations [2,5,24]. This technique is relatively insensitive, with the lower limit for detection by microscopy of approximately one cancer cell in 200 noncancer cells [25]. In addition, it is not unusual to examine less than 0.1% of available lymph node tissue, resulting in sampling error [4,5,25]. These methodological limitations are most evident when the frequency of post-resection disease recurrence is considered. Stage I and II (pN0) lesions, limited to the bowel wall with no histological evidence of lymph node involvement or extra-intestinal spread, should be amenable to complete surgical excision. However, recurrence rates of 30% in stage I and 50% in stage II have been reported [2,3,5,24]. In stage III, where all the detectable tumor, including involved lymph nodes, is removed, recurrence rates greater than 70% have been described [2,10,12–15,17–19,26,27]. Variability in recurrences in pN0 patients reflects a mixture of true pN0 lesions with stage III or IV lesions undetected by histopathology [2,4,5,12,21,28,29].

Adjuvant therapy improves stage-specific survival in patients with colon cancer

Beyond prognosis, stage determines which patients receive adjuvant therapy. Chemotherapy administered post-resection to patients with stage III colon cancer improves survival and can increase time to recurrence by 40% and overall survival by at least 30% [6,20,30–36]. Furthermore, the introduction of biological targeted therapeutics contributed to dramatic increases in 5-year median and overall survival in stage IV patients, from approximately 7% to more than 30% [37]. However, the utility of adjuvant chemotherapy in patients with pN0 colon cancer remains uncertain, with marginal survival benefits in stage II patients in some, but not all, clinical trials [2,3,6,9,20,22,23]. This uncertainty of treatment benefit is echoed in the dynamic evolution of treatment guidelines, in which adjuvant therapy has become discretionary in pN0 patients with clinicopathologic features of poor prognostic risk [9,38–40]. It is tempting to speculate that heterogeneous responses to therapy in pN0 patients reflect, in part, heterogeneity of occult nodal metastases [4,5,21,24,41–43]. Thus, improved methods to detect clinically prognostic occult metastases may better identify pN0 patients who could benefit from adjuvant therapy [6,37].

Molecular diagnostics & staging

While histology remains the gold standard for staging, reflecting the prognostic and predictive relationship between tumor cells in lymph nodes and outcomes [1–6,9,20–23], this approach underestimates metastases. Up to 70% of tumor-containing lymph nodes harbor metastases that are smaller than 0.5 cm and often escape detection by routine techniques [2,3,5,24]. Beyond histology, emerging technologies, including quantitative reverse transcriptase (qRT)-PCR, may provide the most sensitive and specific assessment of metastases [5,24]. Indeed, molecular staging offers an advantage, with the ability to sample the entire specimen and detect one tumor cell in approximately 10^7 normal cells [5,24]. While studies of the staging utility of RT-PCR have yielded heterogeneous results, reflecting inadequate sample size, absent clinical follow-up and variable analytic techniques, meta-analyses support the prognostic value of occult metastases detected by RT-PCR in pN0 patients with colorectal cancer [4,5,21,29,43,44].

GUCY2C as a molecular marker for colorectal cancer

Guanylyl cyclase C (GUCY2C), one of a family of proteins synthesizing cyclic GMP, is selectively expressed by intestinal cells [45–54]. GUCY2C is the receptor for the paracrine hormones guanylin and uroguanylin, whose interaction with the extracellular domain activates the cytoplasmic catalytic domain, inducing cGMP accumulation [50,53,55–61]. GUCY2C regulates homeostasis, coordinating proliferation, DNA repair, metabolic programming and epithelial–mesenchymal interactions organizing the crypt–surface axis [62–74]. In this context, guanylin and uroguanylin are gene products universally lost early in colorectal cancer [75–79]. Moreover, GUCY2C silencing in mice increases tumorigenesis, reflecting dysregulation of the cell cycle and DNA repair [65]. These observations suggest a hypothesis in which GUCY2C is a tumor-suppressing receptor coordinating homeostasis whose silencing through hormone loss contributes to tumorigenesis [63–66,70]. Of significance, GUCY2C was detected in over 1000 samples of normal intestine, but not in more than 1000 extra-gastrointestinal tissues [41,44,46,47,55]. In addition, GUCY2C protein ($n > 200$) and/or mRNA ($n > 900$) were detected in nearly all primary and metastatic human colorectal tumors regardless of anatomical location or grade, but not in extra-gastrointestinal tumors (>200) [41,44,46,47,55,78,80–83]. Furthermore, GUCY2C is overexpressed, at mRNA and protein levels, by more than 80% of colorectal tumors [80,84,85]. Expression normally restricted to intestinal cells but universal overexpression by metastatic colorectal cancer cells underscores the utility of GUCY2C as a molecular marker for colorectal cancer [43].

Prospective validation of GUCY2C as a molecular marker for occult metastases

Retrospective studies suggested that GUCY2C expression detected by RT-PCR was associated with the risk of disease recurrence in colorectal cancer patients [44]. These preliminary observations formed the basis for an adequately powered, prospective, blinded clinical trial of the utility of GUCY2C qRT-PCR to detect prognostically significant occult metastases in regional lymph nodes employing an analytically validated assay platform. When completed, this trial provided level 1 evidence of the utility of qRT-PCR for identifying prognostic occult lymph node metastases [86]. Specifically, these studies compared staging of patients with colorectal cancer by GUCY2C qRT-PCR with conventional histopathology, compared the predictive value of staging by GUCY2C qRT-PCR and histopathology for recurrent colorectal cancer, and developed a predictive model for colorectal cancer recurrence incorporating GUCY2C qRT-PCR as an independent prognostic marker of risk.

Innovations in molecular diagnostics enabling prospective validation

Prospective validation of GUCY2C as a molecular marker for staging patients presented substantial unanticipated analytical challenges reflecting the emerging nature of qRT-PCR for the detection of clinically important biomarkers in large-scale clinical trials. Thus, these studies required an analytically validated assay to quantify GUCY2C mRNA reliably across over 5000 specimens. Moreover, there is an underappreciated principle in qRT-PCR in which the validity of transcript quantification relies on the comparability of individual reaction efficiencies, a parameter that substantially varies reflecting individual patients, biosamples and reactions. To reliably quantify and compare GUCY2C expression across approximately 20,000 qRT-PCR reactions for clinical utility, a technique was required to adjust individual reaction efficiencies.

qRT-PCR assay validation

In the context of analyzing approximately 5000 tissue specimens in a prospective trial, the assay for quantifying GUCY2C mRNA by qRT-PCR was subjected to analytic and clinicopathologic validation [84]. Analytic performance characteristics of the assay were defined employing GUCY2C complimentary RNA standards. Linear mixed-model analysis of the relationship between threshold cycle and GUCY2C cRNA concentration yielded an average intercept of 42.36 (95% CI: 41.94–42.79), average slope of −3.53 (95% CI: −3.62 to −3.44), and a mean amplification slope efficiency of 92%. This assay was linear from 2.5×10^1 to 2×10^6 copies, with a limit of quantification of 25 copies. Overall plate-to-plate variability (CV) was 1%, while within-plate variability was less than 5% at all cRNA concentrations. These performance characteristics were stable in different biological matrices, including human lymph nodes. Clinicopathologic performance of the assay was defined with RNA from lymph nodes with confirmed metastases (true positives) and from patients without colon cancer (true negatives). True-negative (164) lymph nodes exhibited median GUCY2C copy numbers that were less than or equal to 50, while true-positive (15) lymph nodes exhibited median copy numbers greater than 1000. Receiver-operator analysis revealed a nominal sensitivity of 93% and specificity of 97%. These favorable analytic and clinicopathologic characteristics of the GUCY2C qRT-PCR assay suggest its suitability for examining the utility of the marker for staging patients with colorectal cancer.

Efficiency-adjusted relative qRT-PCR

During PCR with the TaqMan® probe technology, DNA or mRNA templates are enzymatically replicated at each cycle of the reaction so that copies created in any cycle emit a fluorescence signal proportional to the number of templates present. For each RT-PCR reaction (one sample in one well on a plate), the fluorescence signal is measured after each PCR cycle. With the cycle number, fluorescence measures constitute kinetic RT-PCR data, which records amplification history for each RT-PCR reaction. An ideal RT-PCR reaction would be described by an exponential growth model with an exponential base of 2. In reality, not all templates are duplicated during the course of the RT-PCR reaction and the proportion of templates that produce copies at each cycle is known as the amplification efficiency. Indeed, this is one key issue in RT-PCR quantification because many reactions do not have ideal or similar efficiencies, although comparisons of results in different reactions presume equivalent efficiencies. Thus, inaccurate and variable estimation of GUCY2C expression could efface true differences reflecting occult metastases. We developed a four-parameter logistic model for fluorescence measures, which are directly proportional to the number of target templates, providing a new method for efficiency-adjusted relative RT-PCR quantification based on estimates from the parameterized logistic model fitted to the full (unbiased) kinetic data from each individual RT-PCR reaction [43,87]. Using qRT-PCR analyses generated in the analytical validation study [84], the efficiency-adjusted relative RT-PCR quantification based on estimates from the parameterized logistic model fitted to the full kinetic data provides more

accurate and precise estimates of individual PCR reaction efficiencies than traditional efficiency estimates based on exponential growth models. Indeed, traditional exponential growth models provided up to fivefold greater variability and sixfold greater empiric bias in normalized estimates of GUCY2C expression, compared with the parameterized logistic model. Moreover, approximately 80% of individual RT-PCR reactions for GUCY2C or the reference gene β-actin provided insufficient exponential growth phase (<four cycles) to apply traditional models for efficiency adjustments, suggesting that most reactions would be uninformative by traditional algorithms. This new method for efficiency-adjusted relative qRT-PCR based on logistic models minimizes bias and variability, maximizes precision and accuracy, and preserves the integrity of information available from all reactions. Importantly, it readily accommodates estimation of target gene expression relative to reference genes using replicate reactions. In the context of these advantages, this technique was applied to analyze GUCY2C expression to detect prognostic occult metastases in lymph nodes.

Prospective analysis of GUCY2C qRT-PCR for staging patients with colorectal cancer

Study description

This study was a prospective multicenter clinical trial. Investigators and clinical personnel were blinded to results of molecular analyses, while laboratory personnel and analysts were blinded to patient and clinical information [43]. To have at least 80% power to detect a hazard ratio of 1.6 ($p \leq 0.05$, two-sided), an established threshold for stage-specific risk stratification [88], 225 pN0 patients were required.

Study population

Between March 2002 and June 2007, 273 stage 0–II pN0 and 87 stage III pN1 colorectal cancer patients were enrolled at one of seven academic medical centers and two community hospitals in the USA and Canada [43]. Patients were ineligible if they had a previous history of cancer, metachronous extraintestinal cancer or perioperative mortality associated with primary resection.

Analytic approaches

Pathology—Lymph nodes and, when available, tumor specimens (51%), were dissected from colon and rectum resections and frozen at −80°C within 1 h to minimize warm ischemia. Half of each resected lymph node was fixed with formalin and embedded in paraffin for histological examination. Specimens from pN0 patients were subjected to molecular analysis if tumor samples, where available, expressed *GUCY2C* mRNA above background levels in disease-free lymph nodes (≥30 copies); and second, at least one lymph node was provided that yielded RNA of sufficient integrity [84]. Thus, *GUCY2C* in tumors was below background levels in 14 patients who were excluded from analysis [84]. Analysis of the 2656 lymph nodes available from the remaining 259 pN0 patients revealed 86 yielding RNA of insufficient integrity by β-actin qRT-PCR, excluding two additional patients [84]. Overall, the 257 pN0 patients who were eligible provided 6699 lymph nodes (range: 2–159; median: 21 lymph nodes/patient) for histopathologic examination, of which 2570 nodes (range: one to 33; median: eight lymph nodes/patient) were eligible for analysis by qRT-PCR. Greater numbers of lymph nodes available for histology compared with molecular analysis from pN0 patients includes those collected after formalin fixation or those less than 5 mm in diameter, smaller than the limit for accurate bisection of fresh tissue.

Molecular—*GUCY2C* and *β-actin* mRNA was quantified using qRT-PCR by the analytically validated assay [84] employing logistic regression of amplification profiles from individual RT-PCR reactions, providing an efficiency-adjusted relative quantification [87].

Statistical—In the absence of established methodologies to define optimal cutpoints for molecular markers from incomplete and variable lymph node collections from individual patients, it was established *a priori* that nodes in which relative *GUCY2C* mRNA was greater than or equal to the overall median would be considered pN0(mol+), while those less than the median would be considered pN0(mol−) [43]. Patients were considered pN0(mol+) if one or more nodes were positive. The primary clinical end point was time to recurrence, measured from date of surgery to time of last follow-up, recurrence event or death [89]. Disease-free survival, defined as time from surgery to any event regardless of cause, was a secondary outcome [89]. Date of recurrence was established by radiography, laboratory studies, physical examination and/or histology. Simultaneous prognostic effects of parameters, including T stage, grade, tumor location, lymphovascular invasion, chemotherapy, total lymph nodes harvested and pN0 molecular status [3] were estimated employing Cox regression analysis. The multivariable model for each outcome included all established prognostic measures, regardless of significance, to establish the additional independent prognostic effect of molecular status.

Observations from prospective analyses of GUCY2C qRT-PCR

Occult metastases & disease recurrence

Quantitative RT-PCR analysis revealed that GUCY2C expression, indicating the presence of occult metastases, was detected in at least one lymph node from 225 patients (87.5%) with pN0 colorectal cancer [43]. These data suggest that, unexpectedly, the majority of patients staged as node-negative by conventional histopathology harbor occult metastases. In that context, the working hypothesis suggests that staging based on GUCY2C qRT-PCR should more accurately predict colorectal cancer recurrence at follow-up than histopathology. Specifically, patients who are pN0(mol+) by GUCY2C qRT-PCR are at greater risk for recurrent disease than patients who are pN0(mol−). Indeed, with a median follow-up of 24.0 months (range: 1.8–62.7) for pN0(mol+) patients and 35.9 months (range: 2.5–62.1) for pN0(mol−) patients, 20.9% of patients with (CI: 15.8–26.8%), but only 6.3% without (CI: 0.8–20.8%) occult metastases developed recurrent disease ($p = 0.006$) [43]. Both GUCY2C-negative patients who developed recurrent disease provided two or fewer lymph nodes for analysis by qRT-PCR, perhaps reflecting the requirement, by any staging technique, for adequate lymph node sampling [2,3, 90,91]. Exploratory analyses suggested that GUCY2C expression conferred a worse prognosis among stage I and II patients and those with colon and rectal cancer. Moreover, occult metastases were associated with reduced disease-free survival in patients, with tumors of different stages and locations. Time to recurrence and disease-free survival in pN0(mol+) patients were comparable to that of patients with stage III pN1 (stage IIIA + IIIB) disease, all of whom have histopathology-detectable metastases in lymph nodes [43].

GUCY2C as a prognostic variable

The working hypothesis suggests that GUCY2C qRT-PCR should enhance multivariable analyses incorporating established prognostic indicators to improve the prediction of prognostic risk. Cox proportional-hazards analyses revealed that T stage, grade, tumor location, lymphovascular invasion, therapy and total lymph nodes harvested were poor as prognostic factors. By contrast, GUCY2C expression provided the strongest independent prognostic information, and patients who were pN0(mol+) exhibited earlier time to recurrence (absolute event rates: pN0[mol−] 6.3%, pN0[mol+] 20.9%; hazard ratio: 4.66 [1.11–19.57]; $p = 0.035$)

and reduced disease-free survival (absolute event rates: pN0[mol−] 12.5%, pN0[mol+] 26.2%; hazard ratio: 3.27 [1.15–9.29]; p = 0.026) [43].

GUCY2C qRT-PCR for detecting occult metastases in pN0 colorectal cancer patients

Prospective detection of occult metastases by GUCY2C qRT-PCR was an independent prognostic marker of risk. Molecular staging revealed that approximately 13% of pN0 patients were free of tumor cells, while approximately 87% had GUCY2C results that suggested occult metastases. However, while a high proportion of pN0 patients exhibit occult metastases by GUCY2C, most pN0 patients will not recur [2,3]. Indeed, as a comparison, not all stage III patients, all of whom have histology-detectable lymph node metastases, ultimately develop recurrent disease [2,3]. Reconciliation of this apparent inconsistency relies on the recognition that nodal metastases, regardless of methods used to detect them, do not assure recurrence but, rather, indicate risk. In support of this concept, this analysis suggests recurrence rates for pN0 (mol+) patients with occult metastases that are nearly identical to those for stage III pN1 patients [2], the lowest stage in which all patients have histology-detectable metastases [1,2]. This analysis is the first to demonstrate the utility of molecular analysis to detect prognostic occult metastases in lymph nodes in an adequately powered, prospective trial with sufficient longitudinal follow-up employing analytically validated assays. Indeed, the absence of this level of evidence has been one limitation to the translation of these paradigms to patient management [4,5]. These considerations underscore the importance of validation with independent cohorts to con-firm the prognostic utility of GUCY2C qRT-PCR in colorectal cancer. There is a well-established relationship between tumor burden, quantified as the number of lymph nodes harboring tumor cells by histopathology and prognostic risk in colorectal cancer patients. Assuming there are adequate numbers of nodes to review [2,3,90, 91], stage III patients with four or more involved lymph nodes exhibit a recurrence rate that is approximately 50–100% greater than those with three or fewer involved nodes [2,3]. As in histology-based analyses, one limitation of our prospective trial was the variable number of lymph nodes available for molecular staging from individual patients. In addition, lymph nodes less than 5 mm were excluded, reflecting size limits for fresh tissue bisection, although they are a rich source of tumor metastases [92,93]. These considerations suggest that the precision of staging by molecular analyses will benefit from optimum lymph node sampling to incorporate tumor burden into prognostic risk stratification [4,5,21]. Our working hypothesis suggested that there is an inverse relationship between the number of lymph nodes that contain occult metastases and risk. Specifically, it was hypothesized that patients with a greater number of lymph nodes containing occult metastases would have a greater prognostic risk compared with patients with a smaller number of involved lymph nodes. We studied the subset of pN0 patients who provided 12 or more lymph nodes for GUCY2C analysis, then applied standard American Joint Committee on Cancer definitions for pN1 and pN2 [2,3]. This analysis revealed that individuals with 0–3 involved nodes exhibited a prognostic risk similar to pN0(mol−) patients (5.9 vs 8.3%) [43]. Conversely, those with four or more involved nodes exhibited a risk ($\leq$3 vs $\geq$4; p = 0.027) identical to patients with stage III pN1 disease [43]. Improved risk stratification by integrating occult metastases and estimates of tumor burden underscores the importance of adequate lymph node sampling for optimum molecular [4,5,21], as well as histological [2,3,90,91], staging in colorectal cancer.

Beyond the number of involved lymph nodes, there is an evolving relationship between the volume of cancer cells in individual nodes, tumor burden and prognostic risk [2,94]. While metastatic foci greater than or equal to 0.2 mm are associated with increased disease recurrence, the relationship between individual tumor cells or nests less than 0.2 mm, and risk remains undefined [2]. The emergence of quantitative RT-PCR provides an unprecedented opportunity

for identification of metastases in tissues. Of significance, the superior sensitivity of qRT-PCR [95], with its optimum tissue sampling and capacity for single-cell discrimination, may identify occult cancer cells in lymph nodes below the threshold of prognostic risk [2], limiting the specificity of molecular staging [43]. In that context, the prospective study was not designed to identify a quantitative threshold defining risk. Indeed, one limitation of that study was the requirement to define *a priori* the diagnostic threshold for GUCY2C. In the future, it will be essential to define the quantitative relationship between marker expression and disease risk that incorporates estimates of tumor burden to optimize prognostic sensitivity and specificity [43].

Expert commentary & five-year view

Historically, the most important prognostic marker of survival and predictive marker of response to adjuvant chemotherapy in colorectal cancer is the histologic detection of tumor cells in lymph nodes [1–6,9,20–23]. Despite its importance, techniques that assess nodal metastases remain imperfect and approximately 30% of patients with pN0 colorectal cancer die of recurrent disease, reflecting occult metastases that escape detection by standard techniques [2–5,21,24,41,42,96]. These observations underscore the unmet clinical need for novel, improved approaches to more accurately assess occult metastases in lymph nodes in patients with colorectal cancer. We have completed a prospective, multicenter, blinded clinical trial that demonstrated the utility of GUCY2C qRT-PCR lymph node assessment to predict recurrence risk [43]. Indeed, occult metastases identified by GUCY2C qRT-PCR was the strongest independent prognostic marker of recurrence risk in pN0 patients, providing the first level 1 evidence to support the hypothesis that prognostic risk in pN0 colorectal cancer is associated with occult metastases [86]. These observations highlight the potential utility of GUCY2C qRT-PCR for staging patients with pN0 colorectal cancer. Translation of these initial observations into useful staging paradigms in colorectal cancer will require a number of critical analyses performed over the next 5 years in studies requiring fewer than 1000 patients:

- Studies will need to validate, in a new patient cohort, the prognostic utility of GUCY2C qRT-PCR for categorical identification (yes/no) of occult metastases as a marker of disease recurrence. This approach is responsive to the emerging learn–confirm paradigm in biomarker translation, in which clinical integration requires validation in independent populations [97–104];
- The superior sensitivity of qRT-PCR [95], with its optimum tissue sampling and capacity for single-cell discrimination, may identify occult cancer cells in lymph nodes below the threshold of prognostic risk [2], limiting the specificity of molecular staging [43]. This is reflected in the detection of occult metastases in 87% pN0 patients, most of whom will not develop recurrent disease [2]. There is an emerging paradigm that goes beyond the categorical (yes/no) presence of tumor cells, to quantify metastatic tumor burden (how much) to more accurately stratify risk. In that context, qRT-PCR provides a unique opportunity to quantify occult tumor burden across the regional lymph node network to establish prognostic risk in pN0 patients;
- Beyond prognosis, there is a well-established relationship between lymph node metastases and chemotherapeutic benefit in patients with colon cancer. While treated stage III patients experience improved survival outcomes, there is uncertainty about the benefits of adjuvant chemotherapy in pN0 patients [2,3,6,9,20,22,23]. It is tempting to speculate that the heterogeneity of observed benefit of therapy in pN0 patients reflects, in part, inaccurate staging [4,5,21,24,41–43]. In our prospective trial, GUCY2C analysis identified a subset of pN0 patients whose clinical behavior paralleled that of stage III patients, staged by traditional criteria. Typically, such patients receive adjuvant chemotherapy suggesting that if pN0 patients at similar risk

could be identified, they too might benefit from adjuvant chemotherapy. Future studies will define whether occult lymph node metastases detected by GUCY2C qRT-PCR is a predictive marker of chemotherapeutic benefit. These studies will determine whether, among patients with occult lymph node metastases, those who receive chemotherapy have better clinical outcomes than those who do not.

Upon successful completion of these studies, the GUCY2C diagnostic paradigm will be closer to assuming a role in lymph node evaluation in pN0 colon cancer patients.

Key issues

- Traditional paradigms for staging patients with colorectal cancer incorporating standard histopathological assessment of regional lymph nodes underestimate the extent of metastatic disease, reflected by 25–30% of pN0 patients developing recurrent disease.
- Limitations of traditional staging paradigms, including volume of tissue assessed and analytic sensitivity, can be eliminated by employing disease-specific markers and a powerful molecular amplification technology such as qRT-PCR.
- GUCY2C identifies metastatic colorectal cancer cells in extra-intestinal tissues, and occult lymph node metastasis detected by GUCY2C qRT-PCR is an independent prognostic indicator for risk of disease recurrence in pN0 colorectal cancer patients.

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