6

Multigene Expression Biomarkers and Score Systems for Predicting Therapeutic Benefit in Gastrointestinal Cancers

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Abstract: Gastrointestinal cancer is a leading cause of death among cancer patients worldwide. For both gastric and colorectal cancers, the 5-year overall survival for advanced stages remain low. Their polygenic and heterogeneous nature is characterized by alterations in multiple molecular pathways throughout its development, which is a big challenge for patient risk stratification and for treatment options. In this chapter, we describe the development of prognostic and predictive multigene signatures in gastrointestinal cancer patients for clinical use. We identified and validated a novel 53–gene prognostic signature and score system that robustly and reliably predicts overall survival in gastric cancer patients. We also discovered that the predictive potential of the 53-gene signature that can

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identify gastric cancer patients who may benefit from adjuvant FOLFOX chemotherapy. In addition, we developed a 15-gene signature with robust prognostic function in colorectal cancers. Both signatures are independent of molecular subtypes and clinical outcomes. The predicting capability of these signatures supersedes previously published prognostic signatures in the same types of cancers. For clinical application, we developed a nucleic acid hybridization-based gene expression assay for the signatures. Future prospective studies are warranted to test the clinical value of these multigene signatures and fully deploy them into patient use.

Keywords: colorectal cancer; gastric cancer; multigene expression assay for gastrointestinal cancers; multigene expression biomarkers for gastrointestinal cancers; overall survival

INTRODUCTION

Worldwide, gastrointestinal cancers represent more than one-quarter of the cancer incidence and over one-third of all cancer-related deaths (1). Currently, curative surgery with adjuvant chemotherapy is the most common treatment design for stage II–III gastric cancer and stage III colorectal cancer. Despite some improvements in recent years, the 5-year overall survival for advanced stages of gastrointestinal cancers remains low (below 30% for gastric cancer and about 14% for stage IV colorectal cancer). This may be contributed by many factors including genetic, histopathological, and clinical variations among patients. Therefore, it is a big task to identify those factors with critical and independent value for predicting patient clinical outcome for a more accurate personalized risk assessment for treatment decisions.

Both gastric cancer and colorectal cancer are polygenic disorders with variable responsiveness to treatment such as chemotherapy and immunotherapy, as observed in clinical practice. Recent comprehensive omics studies with microarray technology and next generation sequencing/genome wide association studies have unveiled vast genomic information and many heterogeneous features of the two diseases (2–5). For example, four molecular subtypes have been identified in gastric cancer (Epstein-Barr virus (EBV)-positive, microsatellite unstable, genomically stable and chromosomal instability) (5) and colorectal cancer (microsatellite instability, genome stable, chromosomal instability, and hypermutated-single nucleotide variant) (6) through comprehensive molecular profiling using The Cancer Genome Atlas (TCGA). Such classification reflects both background genetics and molecular pathogenetic features. However, new biomarkers are needed to identify gastrointestinal cancer patients for susceptibility toward the clinical therapies.

To bridge this gap, genomic biomarkers have increasingly been developed and utilized in recent years, to stratify patients and predict clinical outcome, for instance, being used as prognostic and predictive biomarkers in various types of cancers (7–10). Such genomic assays on predicting clinical outcome may aid physicians in determining a most suitable clinical therapy for the patient, as effectively shown in the breast cancer with FDA-approved Oncotype DX (8) and MammaPrint (7) tests.

In gastrointestinal cancers, numerous reports on gene expression patterns have been published to predict patient outcomes such as recurrence, metastasis, and benefit from adjuvant therapies (11–18). However, to the best of our knowledge, extended validation of bioinformatics findings is rare with such biomarker signatures, and these have not yet been clinically implemented, except for the 7-gene *Oncotype* DX colon cancer test for the prediction of recurrence risk for stage II and III colorectal cancers (19, 20).

In this chapter, we describe the development of prognostic and predictive multigene signatures in gastrointestinal cancer patients for clinical use. We first identified a novel 53–gene prognostic signature and score system that robustly and reliably predicts overall survival in gastric cancer patients (12), which were validated in multiple centers (16). We also discovered the predictive potential of the 53-gene signature that can identify gastric cancer patients who may benefit from adjuvant FOLFOX (leucovorin calcium, fluorouracil, and oxaliplatin) chemotherapy (16). Later, we developed a 15-gene signature with robust prognostic function in colorectal cancer (21). Both signatures are independent of molecular subtypes and clinical outcomes. The predicting power of these two signatures supersedes previously published prognostic signatures in the same types of cancers. For clinical application, we developed a nucleic acid hybridization-based gene expression assay for the signatures and successfully employed it in a multiple hospital-based retrospective cohort study (16). Effective translation of laboratory findings into medical practice depends on both their clinical implications and assay development.

PROGNOSTIC/PREDICTIVE MULTIGENE EXPRESSION SIGNATURE DEVELOPMENT IN GASTROINTESTINAL CANCERS

The research interest in our laboratories has been in identifying distinct subsets of cancer patients with prognostic and/or predictive outcomes for precision medicine. We developed a novel multi-step bioinformatic analytic strategy to identify robust multi-gene expression prognostic/predictive signatures and to build related scoring systems for clinical use. Figure 1 shows how to mine publicly available omics data and associated clinical information, e.g., TCGA (RNA-sequencing data) and Gene Expression Omnibus (NCBI- GEO) (microarray-based), followed by a canonical discriminant analysis to establish a 53-gene expression signature. In the process, the Kaplan-Meier method together with Cox regression analysis was used to evaluate association of the gene expression levels with patient overall survival in gastric cancer (12, 16).

In addition to the gastrointestinal cancers, we have also successfully established a 27-gene panel for lung adenocarcinoma (22), and a 11-gene panel for ovarian cancer (23). In such cases, we first demonstrated that these expression-based signatures were able to better predict prognosis in comparison with other already published multigene signatures using the same datasets, as described in detail below, which clearly indicate that our strategy has its own advantage. As a matter of fact, this is the most important step, otherwise there is no meaning to

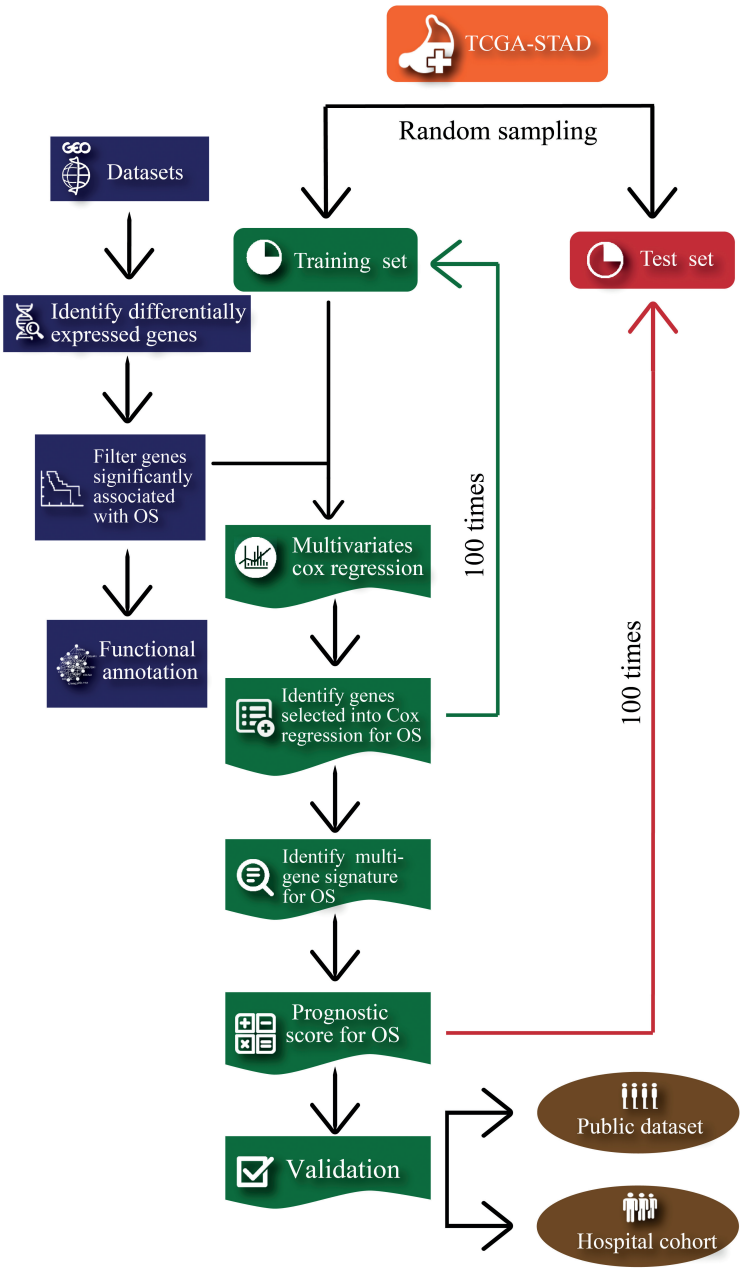

Figure 1. A working flowchart for the identification, development, and validation of a prognostic multigene signature (using gastric cancer as an example). Left panel: screening of consistently unregulated genes in gastric cancer tissue using meta-analysis and identification of overall survival-associated genes with Kaplan–Meier analysis using log-rank testing. Right panel: the steps to develop a 53-gene expression based prognostic signature and score system using TCGA-STAD.

develop a multigene biomarker that is inferior in predicting ability to other already published ones.

Next, to check whether the prognostic impact of a signature is independent of any potential confounding factors, we usually perform univariate and multivariate Cox regression analysis on all available clinicopathological parameters that may affect the prognostic capability. In addition, we examine and rule out any significant correlation of our signature with molecular subtypes of the same cancer type. For example, we investigated whether the prognostic value of the 53-gene and 15-gene signatures would be enhanced in certain molecular subtypes of gastric cancer or colorectal cancer, as such subtypes are associated with different survival outcomes and treatment benefits.

The validation of a newly established signation is usually carried out using two different sets of data: (i) transcriptome data from publicly available independent datasets; and (ii) collection of patient samples to perform gene expression assay in a cohort study, ideally a multicenter based one.

For a successful clinical application, it is essential to develop a reliable, sensitive, and high-throughput gene expression assay in suitable patient samples. RT-qPCR, as a mature technology, is routinely used to quantify mRNA levels of prognostic genes in clinical settings, as best demonstrated in the 21-gene Oncotype DX assay (7, 8). We recently developed a modified RNA hybridization assay using routinely prepared formalin fixed paraffin-embedded (FFPE) specimen, for the quantitative measurement of mRNA (16), which offers a 96-well high-throughput platform. This technique, as reported before, could be more reliable than RT-PCR to detect RNA or DNA signal in archived FFPE samples (24–26).

Another type of multigene prognostic/predictive biomarkers is based on a group of genes with similar functions. One example is using a panel of DNA repair genes to predict therapeutic responses in cancer patients. For example, as recent evidence revealed, DNA repair landscape is a significant factor in driving response to immune checkpoint blockade therapy (27). We developed a novel 15-DNA repair gene signature (DRGS) and scoring system to evaluate its efficiency in discriminating different molecular and immune characteristics and therapeutic outcomes of patients with gastric cancer (28). Multi-omics data analysis demonstrated that the patients with high DRGS score were characteristic of high levels of antitumor lymphocytes infiltration, tumor mutation burden (TMB) and PD-L1 expression, and such patients exhibited a longer overall survival and may benefit more from immune checkpoint blockade therapy, as compared to the low-score patients (28). Therefore, the DRGS and its scoring system may have implications in tailoring immunotherapy in gastric cancer.

53-GENE PROGNOSTIC ASSAY IN GASTRIC CANCER

In 2016, to test the hypothesis that tumor-specific genetic features of gastric cancer are a key driver of tumor outcome, which can be utilized to establish prognostic scoring to improve prediction of overall survival of gastric cancer patients, we analyzed differential gene expressions in gastric cancer using publicly available databases. We first identified 276 genes that were robustly differentially expressed between normal and gastric cancer tissues in TCGA gastric adenocarcinoma

cohort (TCGA-STAD) and NCBI- GEO (GSE30727), of which, 249 genes were discovered to be significantly associated with overall survival by univariate Cox regression analysis (12). Functional annotation studies showed that significant enrichment of these genes in cell cycle, RNA/ncRNA process, acetylation and extracellular matrix organization. Finally, a 53-gene signature was established, and a prognostic scoring system developed based on a canonical discriminant function of 53 genes and successfully applied it to predict overall survival of gastric cancer patients in the TCGA gastric adenocarcinoma cohort (TCGA-STAD) as well as in the GSE15459 dataset (Figure 2) (12).

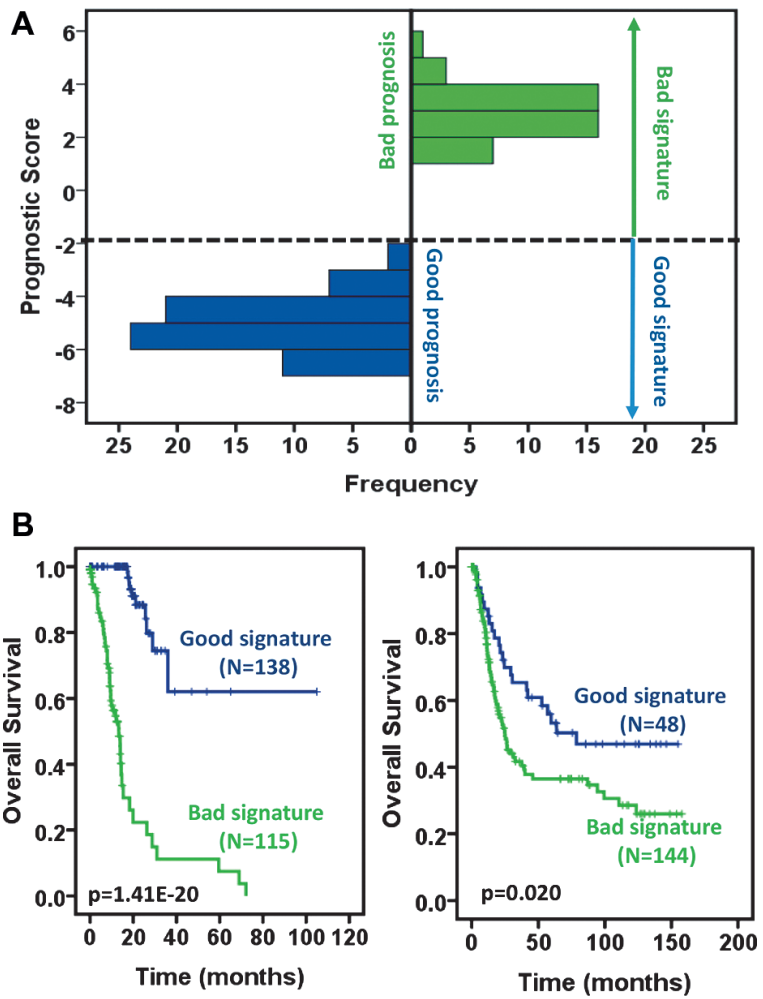

Figure 2. Development of a 53-gene prognostic scoring system for gastric cancer patients. **A.** Distribution of prognostic score between patients with good and bad prognosis in the TCGA data. **B–C.** Prognostic scores are significantly associated with overall survival of gastric cancer patients in TCGA (B) and GSE15459 (C) as demonstrated by Kaplan-Meier survival curves. The p values were obtained from a log-rank test between two groups. This figure is taken from reference 12 with permission.

Using cross-validation approach combined with a multivariate Cox regression analysis, we evaluated and compared the performance of our 53-gene signature with three other published multigene models (13–15) in TCGA-STAD. We discovered that the differences between our signature and any of the other three signatures were significant for both the "intermediate *vs.* good" and "poor *vs.* good" groups (p<0.0001) (Figure 3A), indicating that the 53-gene score

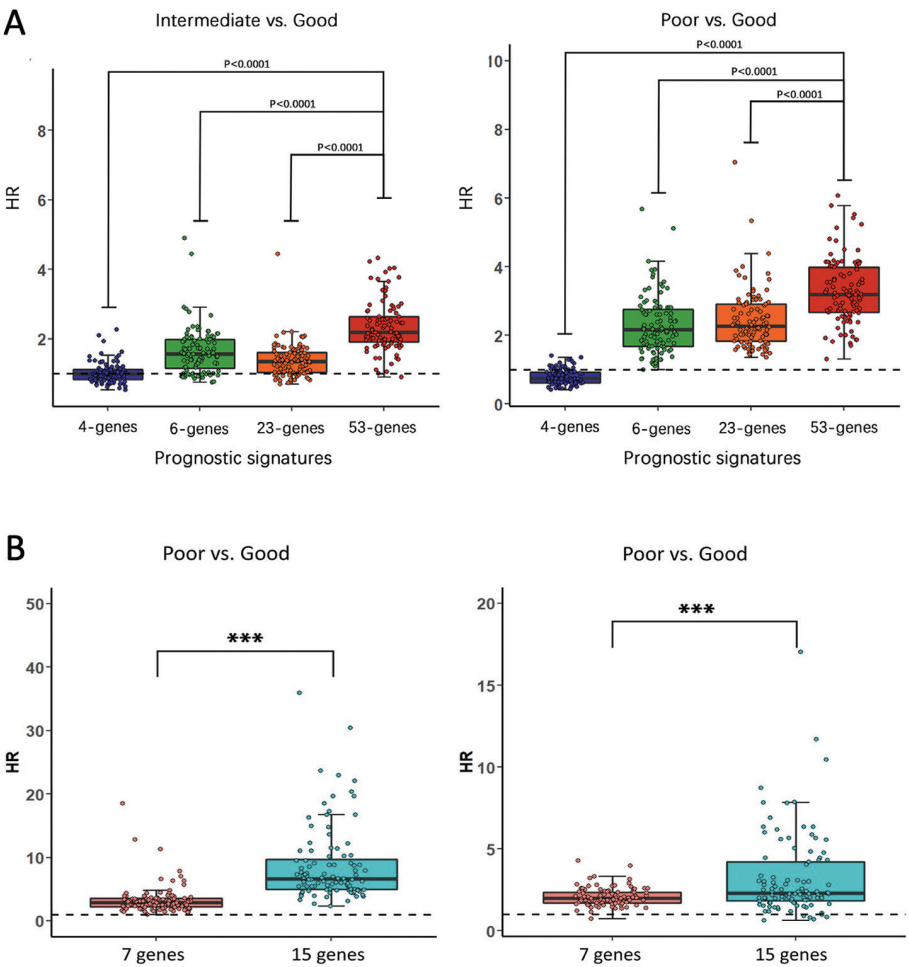

Figure 3. Comparison of the prognostic performance of our signatures with existing prognostic signatures in gastric cancer and colorectal cancer patients. For both signatures, the hazard ratio values of all the 100 test sets were calculated using a Cox model based on the prognostic score between groups. **A.** The differences between the 53-gene signature and other three signatures (13–15) were significant for both the intermediate vs. good and poor vs. good groups (p<0.0001, Mann-Whitney U test). This figure is taken from reference 16 with permission. **B.** Comparison of the 15-gene signature with the 7-gene Oncotype DX colon cancer signature. The comparison was made using the hazard ratio values obtained from 100 test sets between our 15-gene signature and the 7-gene panel in GSE17536 (A) and GSE28722 (B). For each of these datasets, the hazard ratio values calculated for poor vs. good were plotted. *** indicates P < 0.001 in Wicoxon test. This figure is taken from reference 21 with permission.

significantly performs better than other signatures in discriminatively determining overall survival of patients with gastric cancer (16).

Following establishing the 53-gene signature, we carried out a retrospective multi-center study and successfully validated the prognostic power of the 53-gene prognostic assay in 540 patients from three hospitals (enrolled between 2008–2013) using a reliable high-throughput mRNA hybridization-based assay (16). To the best of our knowledge, this is the first multi-center clinical study for validating a multi-gene expression signature in a relatively large-sized gastric cancer patient cohort (16). In this study, 180 patents from two hospitals were randomly selected to build a prognostic prediction model based on the 53-gene signature using leave-p-out (one-third out) cross-validation method together with Cox hazard regression and Kaplan-Meier analysis, and then the model was tested in the independent cohorts, a total of 360 patients with stage I–IV gastric cancers.

Multivariate Cox regression analysis demonstrated that the 53-gene signature predicts prognosis in gastric cancer patients independent of clinicopathologic information including age, gender, TNM staging, WHO histologic types and differentiation (16). Therefore, the 53-gene prognostic score is an independent prognostic factor.

One key discovery of this work is that this prognostic signature is also predictive of drug response in gastric cancer patients, when the effect of adjuvant FOLFOX (leucovorin, fluorouracil, and oxaliplatin) chemotherapy and other first-line chemotherapies were compared for patient overall survival in different prognostic score groups (16). The former is the most commonly used chemotherapy for gastric cancer after surgery in the patients enrolled in our study. We found that patients with good score had a significantly better 5-year overall survival rate from FOLFOX regime than those from other chemotherapy plans. As shown in Figure 4, for patients treated with FOLFOX, the 5-year overall survival rate can reach more than 80% in the group with good prognostic scores, which is significantly higher than ~60% in patients underwent other first-line chemotherapies ($P = 0.028$). However, we did not notice significant difference in intermediate and poor score groups between the FOLFOX and other treatment groups. These data indicate that patients with a good score may experience much better benefit

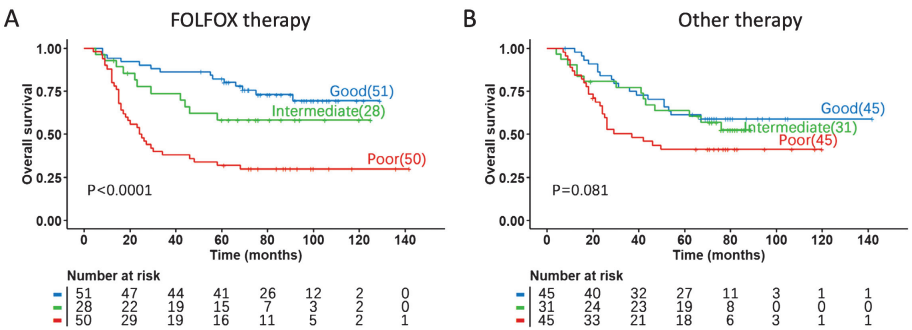

Figure 4. The predictive value of the 53-gene prognostic score in gastric cancer chemotherapy. The Kaplan-Meier curves and P values of overall survival in two different chemotherapy groups were plotted. **A**, Patients with FOLFOX; **B**, Patients with other first-line drugs/regimes. The P-values were obtained by log-rank test. This figure is taken from reference 16 with permission.

from FOLFOX chemotherapy after gastrectomy as compared with other chemotherapies. Therefore, the 53-gene prognostic signature could be a promising predictive biomarker for FOLFOX regimen.

15-GENE PROGNOSTIC SIGNATURE IN COLORECTAL CANCER

For our gene expression signature development in colorectal cancer, we first identified 738 genes that were consistently deregulated in colorectal cancer versus normal colon tissue in six transcriptome datasets (21). Of them, 78 genes were significantly associated with overall survival of colorectal cancer patients. Next, we utilized the concordance statistics for Cox modeling (29) to further refine the gene set with respect to their goodness-of-fit in survival models and to determine the optimal number of genes in the prognostic signature. The final set of 15 genes demonstrated clear discriminative capability to stratify colorectal cancer patients based on good versus poor prognosis (21).

With Cox regression analysis in two datasets, we compared the prognostic power of the 15-gene signature with the 7-gene panel in the *Oncotype* DX Colon Test. As shown in Figure 3, in the two GEO colon cancer datasets used, the median hazard ratio of our signature for poor versus good outcomes was 2.32- and 1.58-fold higher, respectively, as compared to the 7-gene signature, indicating that the 15-gene signature outperforms the 7-gene panel in predicting the overall survival of colorectal cancer patients.

To validate the 15-gene signature in different datasets, we used two datasets, GSE28722 and GSE39582, for Cox regression analysis. We found that high prognostic score patients had a significantly shortened overall survival compared to low score patients. Moreover, the efficacy of this signature was assessed in a retrospective cohort of 203 patients from Nanjing Drum Tower Hospital with stage I or II colorectal cancer. Overall survival analysis demonstrated significantly different survival rates ($P < .0001$ by log-rank test) among the three prognostic score groups in the above Chinese patient cohort (21) with early-stage colorectal cancer. Similar to gastric cancer, we examined whether the prognostic power of this signature is independent of clinicopathological factors potentially associated with patient outcomes in both The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) and our Nanjing Drum Tower Hospital cohort. Data support that the prognostic effectiveness of the 15–gene signature was independent of all the clinical parameters tested, including molecular subtypes ($P < .05$) (21).

CONCLUSION

This chapter is focused on our recent study on developing prognostic and predictive multigene signatures/score systems in gastrointestinal cancer patients in clinical use. In conclusion, we identified and validated in both publicly available databases as well as multi-hospital cohorts a novel prognostic/predictive 53-gene signature that robustly and reliably predicts overall survival in patients with

gastric cancer. We also observed that the predictive potential of 53-gene signature-based score towards the benefit of FOLFOX chemotherapy. We also developed a 15-gene signature with similar functions in colorectal cancer, which was also validated in two independent public datasets and in one hospital. These signatures are independent of molecular classifiers and clinical variables that are associated with patient outcomes. Very critically, our data showed that both the 53-gene and 15-gene signatures supersede previously published prognostic signatures for gastric cancer and colorectal cancer, respectively. The nucleic acid hybridization-based gene expression assay developed is now applicable clinically to assess the overall survival for gastrointestinal cancer patients. For future directions, clinical prospective cohort studies with large patient sizes are warranted to fully deploy these multigene signatures and score systems into clinical use.

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