LETTER TO THE EDITOR

Personalized analysis of minimal residual cancer cells in peritoneal lavage fluid predicts peritoneal dissemination of gastric cancer

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Abstract

Peritoneal dissemination (PD) is a major type of gastric cancer (GC) recurrence and leads to rapid death. Current approaches cannot precisely determine which patients are at high risk of PD to provide early intervention. In this study, we developed a technology to detect minimal residual cancer cells in peritoneal lavage fluid (PLF) samples with a personalized assay profiling tumor-specific mutations. In a prospective cohort of 104 GC patients, the technology detected all the cases that developed PD with 100% sensitivity and 85% specificity. The minimal residual cancer cells in PLF were associated with a significantly increased risk of PD (HR = 145.13; 95% CI 20.20–18,435.79; p < 0.001), which was the strongest independent predictor over pathologic diagnosis and cytological diagnosis. In pathologically high-risk (pT4) patients, the PLF mutation profiling model exhibited a greater specificity of 91% and a positive predictive value of 88% while retaining a sensitivity of 100%. This approach may help in the postsurgical management of GC patients by detecting PD far before metastatic lesions grow to a significant size detectable by conventional methods such as MRI and CT scanning.

Keywords: Gastric cancer, Peritoneal lavage fluid, Peritoneal dissemination, Personalized mutation assay, Minimal residual disease

To the Editor,

Peritoneal dissemination (PD) is a major type of gastric cancer (GC) recurrence and a strong indicator of poor prognosis [1, 2]. Although multiple therapeutic solutions such as hyperthermic intraperitoneal chemotherapy (HIPEC) have been developed to prevent PD [3–5], current diagnostic approaches cannot precisely determine which patients will develop PD. When detectable by CT/MRI or causing symptoms, PD lesions are often of significant size, with no effective treatments available. Minimal residual disease (MRD) detection based on tumor-specific mutations in plasma cell-free DNA (cfDNA) has shown promising performance in prognostic prediction and disease monitoring in several tumor types, including breast, colorectal and lung cancers [6–10].

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Here, we developed customized mutation profiling technology to detect minimal residual cancer cells from peritoneal lavage fluid (PLF). For each case, 20 tumor-specific mutations were selected from exome sequencing of the tumor tissue, and a personalized assay based on Mutation Capsule, a mutation profiling technology, was developed to detect the mutations. The assay was applied to the genomic DNA from cell pellets in the matched PLF samples, which were collected after abdominal exploration and before any manipulation of the stomach in the surgery. A model was developed to determine the fraction of cancer cells among normal cells in PLF based on the number and fraction of the mutations detected (Fig. 1a). The materials and methods are shown in detail in Additional file 1.

To validate the accuracy of the assay, we made a standard reference with serial dilutions of PLC/PRF/5 cells into A549 cells (Additional file 2: Table S1). We selected 20 SNPs unique to PLC/PRF/5 to profile in the genomic DNA of the cell dilutions (Additional file 2: Table S2). We found a strong linear correlation between the theoretical and estimated dilution ratios up to a dilution of 1:100,000 ($R^2 = 0.9998$) (Fig. 1b). Background noise observed at 0% PLC/PRF/5 cell input was <0.0007% among 20 independent replicates, and the assay confidently detected a 0.001% fraction (Fig. 1c, d). To evaluate the biological noise of mutations from nontumor cells in the PLF sample, we profiled 20 mutations that were not detected in the matched tumor sample. We found background noise in all PLF clinical samples to be <0.01%, which was used as the cutoff for MRD detection in the subsequent analysis (Fig. 1e).

We applied MRD analysis to a cohort of 104 GC patients with radical resection (Additional file 3: Figure S1; Additional file 2: Table S3). Based on exome sequencing of tumor tissue, 17 (median, range 3–23) mutations were selected and profiled on matched PLF samples (Additional file 2: Table S4, S5). The cancer cell fraction ranged from 0 to 23.41%, and 42 cases (40%) were MRD-positive. At the 41-month follow-up, 27 out of the 104 patients experienced PD, and all (100%) of them were MRD-positive. Six patients developed lymphatic metastasis, and 4 (67%) were MRD-positive (Fig. 2a, b; Additional file 2: Table S6). In addition, 15% (11/71) of the nonrecurrence patients were MRD-positive. The MRD analysis achieved 100% sensitivity, 85% specificity and 71% positive predictive value (PPV), and the MRD-positive patients showed a high risk of PD (HR = 145.13; 95% CI 20.20–18,435.79; $p < 0.001$) (Fig. 2c, d). In addition, MRD-positive patients were associated with decreased recurrence-free survival (RFS) and overall survival, and all 10 patients who died during the follow-up were MRD-positive patients with disease recurrence (Fig. 2d, e; Additional file 3: Figure S2A).

We further compared the predictive value of the MRD analysis with clinical risk factors (Additional file 3: Figure S2B, C). The MRD analysis showed the best performance (Fig. 2c) and was the strongest independent predictor of RFS for PD based on the Cox proportional hazard model (Additional file 2: Table S7). Interestingly, the MRD analysis exhibited a lower false-positive rate among stage pT4 patients ($n = 56$), delivering 100% (23/23) sensitivity, 91% (30/33) specificity and 88% (23/26) PPV (Fig. 2c, f).

In conclusion, our mutation-based MRD analysis exhibited highly improved performance compared with cytology or other PLF-based assays, making early detection and early intervention far before PDs become clinically detectable possible. This approach may help improve postoperative treatment to prevent PD and improve the overall survival of GC.
Fig. 1 (See legend on previous page.)
Fig. 2 (See legend on next page.)
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Availability of data and materials
The dataset supporting the conclusions of this article is available in the Genome Sequence Archive for Human repository (HRA000528 in https://bigd.big.ac.cn/gsa-human/).

Declarations
Ethics approval and consent to participate
All patients provided written informed consent, and the study was approved by the Ethics Committee of National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (Approval Number: 17-093/1349).

Consent for publication
Not applicable.

Competing interests
Yuchen Jiao, Dongbing Zhao, Pei Wang, Qianqian Song and Pinli Yue have filed patents/patent applications based on the technology and data generated from this work. Yuchen Jiao is one of the cofounders, has owner interest in Genetron Holdings, and receives royalties from Genetron. The remaining authors disclose no conflicts.

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References
1. Badgwell B, Roy-Chowdhuri S, Chiang YJ, Matamoros A, Blum M, Fournier K, et al. Long-term survival in patients with metastatic gastric and gastroesophageal cancer treated with surgery. J Surg Oncol. 2015;111(7):875–81.

2. Thomassen I, van Gestel YR, van Ramshorst B, Luyer MD, Bosscha K, Nienhuijs SW, et al. Peritoneal carcinomatosis of gastric origin: a population-based study on incidence, survival and risk factors. Int J Cancer. 2014;134(3):622–8.
3. Desiderio J, Chao J, Melstrom L, Warner S, Tozzi F, Fong Y, et al. The 30-year experience - A meta-analysis of randomised and high-quality non-randomised studies of hyperthermic intraperitoneal chemotherapy in the treatment of gastric cancer. Eur J Cancer. 2017;79:1–14.

4. Lei Z, Wang J, Li Z, Li B, Luo J, Wang X, et al. Hyperthermic intraperitoneal chemotherapy for gastric cancer with peritoneal metastasis: a multicenter propensity score-matched cohort study. Chin J Cancer Res. 2020;32(6):794–803.

5. Bonnot PE, Piessen G, Kepenekian V, Decullier E, Pocard M, Meunier B, et al. Cytoreductive surgery with or without hyperthermic intraperitoneal chemotherapy for gastric cancer with peritoneal metastases (CYTO-CHIP study): a propensity score analysis. J Clin Oncol. 2019;37(23):2028–40.

6. Coombes RC, Page K, Salari R, Hastings RK, Armstrong A, Ahmed S, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. Clin Cancer Res. 2019;25(14):4255–63.

7. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, et al. Analysis of plasma cell-free DNA by ultradepth sequencing in patients with stages I to III colorectal cancer. JAMA Oncol. 2019;5(8):1124–31.

8. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature. 2017;545(7655):446–51.

9. McDonald BR, Contente-Cuomo T, Sammut SJ, Odenheimer-Bergman A, Ernst B, Perdigones N, et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. Sci Transl Med. 2019. https://doi.org/10.1126/scitranslmed.aax7392.

10. Azad TD, Chaudhuri AA, Fang P, Qiao Y, Esfahani MS, Chabon JJ, et al. Circulating tumor DNA analysis for detection of minimal residual disease after chemoradiotherapy for localized esophageal cancer. Gastroenterology. 2020;158(3):494-505.e6.

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