Traps and trumps from adjacent-to-tumor samples in gastric cancer research

Paulo Pimentel de Assumpção¹, André Salim Khayat¹, Taíssa Maíra Thomaz Araújo¹, Williams Fernandes Barra¹, Geraldo Ishak¹, Aline Maria Pereira Cruz Ramos¹, Sidney Emanuel Batista dos Santos¹, Andrea Kely Campos Ribeiro dos Santos¹, Samia Demachki¹, Paula Baraúna de Assumpção², Danielle Queiroz Calcagno¹, Ney Pereira Carneiro dos Santos¹, Mônica Baraúna de Assumpção³, Fabiano Cordeiro Moreira¹, André Mauricio Ribeiro dos Santos², Carolina Baraúna de Assumpção⁴, Gregory Joseph Riggins⁵, Rommel Mario Rodríguez Burbano⁶

¹Oncology Research Center, Federal University of Pará, Belém 66075-110, Brazil; ²PhD Program in Genetics and Molecular Biology, Federal University of Pará, Belém 66075-110, Brazil; ³Gastrointestinal Endoscopy Service, João de Barros Barreto University Hospital, Belém 66073-000, Brazil; ⁴Department of Surgical Oncology, Oswaldo Cruz Germany Hospital, São Paulo 01327-001, Brazil; ⁵Department of Neurosurgery, Johns Hopkins University, Baltimore 21287, USA; ⁶Laboratory of Molecular Biology, Ophir Loyola Hospital, Belém 66060-281, Brazil

Correspondence to: Paulo Pimentel de Assumpção. Oncology Research Center, Federal University of Pará, Belém 66075-110, Brazil. Email: assumcaopp@gmail.com.

Abstract

The search for cancer biomarkers is frequently based on comparisons between tumors and adjacent-to-tumor samples. However, even after histological confirmation of being free of cancer cells, these adjacent-to-tumor samples might harbor molecular alterations which are not sufficient to cause them to look like cancer, but can differentiate these cells from normal cells. When comparing them, potential biomarkers are missed, and mainly the opportunity of finding initial aberrations presents in both tumors and adjacent samples, but not in true normal samples from non-cancer patients, resulting in misinterpretations about the carcinogenic process. Nevertheless, collecting adjacent-to-tumor samples brings trumps to be explored. The addition of samples from non-cancer patients opens an opportunity to increase the finds of the molecular cascade of events in the carcinogenic process. Differences between normal samples and adjacent samples might represent the first steps of the carcinogenic process. Adding samples of non-cancer patients to the analysis of molecular alterations relevant to the carcinogenic process opens a new window of opportunities to the discovery of cancer biomarkers and molecular targets.

Keywords: Adjacent-to-tumor; trumps; traps

Submitted May 08, 2018. Accepted for publication Aug 23, 2018. doi: 10.21147/j.issn.1000-9604.2018.05.10

Background

The search for cancer biomarkers is frequently based on comparisons between cancer and non-cancer samples (1-4).

Due to practical issues, including easy access and avoidance of inter-individuals differences, the “normal” tissue is usually collected from an area nearby the tumor, and macroscopically free of cancer cells (5-7).

Even after histological confirmation of being free of cancer cells, these adjacent-to-tumor samples might harbor molecular alterations which are not sufficient to cause them to look like cancer, but can differentiate these cells from normal cells (8).

Most of the findings related to gastric carcinogenesis, including the search for new biomarkers, were performed from the comparison of tumor samples and adjacent-to-tumor samples (9-12), and many advances have come from this type of analysis, such as the foundation of the multi-institutional consortia, the Cancer Genome Atlas (TCGA). Currently, strategies for molecular research in cancer, including gastric cancer, have been based on these multi-institutional consortia, favoring extensive investigations...
with relevant sample numbers and patients from different backgrounds, and ensuring more robust and potentially applicable conclusions worldwide (13-17). However, there is a bias arising from the potential occurrence of molecular alterations in the tissue adjacent to the tumor, leading to suboptimal analysis with eventual loss of opportunities for the discovery of biomarkers, since they are expressed in both tissues. On the other hand, there is also an opportunity to explore these undervalued changes favoring knowledge of the initial steps of carcinogenesis.

**Cancer field**

Slaughter et al. (18), aiming to explain the occurrence of multiple cancers among head and neck tumors, proposed the theory of “field effect”, also known as cancerization field or cancer field. Accordingly, exposure to a carcinogenic insult promotes alterations not restricted to the cancer site, but also in the surrounded exposed areas. These alterations in the adjacent-to-tumor cells might evolve, or not, to additional aberrations, and even cancer.

Due to the broad access to high throughput molecular investigations, this field effect theory was re-accessed, deeply evaluated and confirmed (19-21).

Currently, the field effect is widely accepted for many cancer types, and genetic and epigenetic alterations have been strongly demonstrated nearby tumors (22-25).

Additionally, the cancer field might be present with or without morphological alterations, and seems to interact with the surrounding microenvironment, resulting in significant functional modifications, or not (17,26,27).

**Carcinogenic process and cancer diagnosis**

From the first driver mutation to the onset of an invasive cancer, a long period of time is necessary. For the majority of tumors, the carcinogenic process takes over 20 years (28).

Such a long time might enable researchers and physicians to discover cancer, or even stop the process at the initial phases. Nevertheless, the current clinical practice is mainly based on the presence of symptoms and signs of cancer to launch a cancer investigation. In other words, with the exception of few cancer types that are screened, a diagnosis of cancer waits for occurrence of alarm signs such as weight loss, anemia, dysphagia, vomiting, hemorrhage, palpable mass and others to be performed (29-31).

Evidently, in such cases, the diagnosis will be made in very advanced stages, and the treatment outcomes will be invariably poor (32-34).

**Field effect and carcinogenic process**

The field of cancerization encompasses cells exposed to a carcinogenic insult, which is able to provoke diverse molecular alterations (35). Although the majority of such molecular alterations are neutral, or passenger alterations, without relevance to the carcinogenic process, driver mutations can also emerge (36,37).

Even in the case of occurrence of driver mutations, this will rarely evolve to an additional cancer (second primary tumor), since the completeness carcinogenic cascade must be reached (28).

Although not harboring every element of the carcinogenic process, the adjacent-to-tumor samples may present the earliest event of the carcinogenic process, and by this way, should be deeply explored by a different approach from the usual consideration of being a normal control to be compared to cancer samples (15,17).

**Adjacent-to-tumor sample trap**

The conventional practice of using adjacent-to-tumor samples as normal controls, to be compared to cancer samples, aiming to find cancer biomarkers, might be a trap (15).

Molecular alterations presenting in both tumor and adjacent samples will not be identified as “abnormal” (15).

Missing potential biomarkers and mainly the opportunity of finding initial aberrations presenting in both tumors and adjacent samples, but not in true normal samples from non-cancer patients, should be a dramatic loss, resulting in misinterpretations about the carcinogenic process (15).

**Adjacent-to-tumor sample trumps**

Nevertheless, collecting adjacent-to-tumor samples brings trumps to be explored.

The addition of samples from non-cancer patients opens an opportunity to increase the finds of the molecular cascade of events in the carcinogenic process. Differences between normal samples and adjacent samples might represent the first steps of the carcinogenic process.

It should be noted that patients undergoing endoscopy for non-neoplastic causes are usually submitted to multiple gastric biopsies, and although the molecular evaluation of an additional biopsy does not represent an assured benefit to the patient, this biopsy is practically free of additional risks.

Additionally, searching for known driver mutations, presented in both adjacent and cancer samples, could also shed light to the understanding of this pathway, and
provide opportunities to find biomarkers and potential targets to stop the process.

**Triple trumps**

Adding samples from non-cancer patients to the analysis of molecular alterations relevant to the carcinogenic process opens a new window of opportunities to the discovery of cancer biomarkers and molecular targets.

Making comparisons among these three sources of cells might allow elucidation of hidden molecular steps of the carcinogenic process, as is the case of transitions from normal status to the cancer field, and from the cancer field to cancer.

The analysis of alterations presented in both adjacent and cancer cells (and absent in non-cancer patients) instead of being neglected should be addressed, because it might represent initial driver alterations, and potential biomarkers, or also targets for innovative approaches to interfere in the cancer process.

**Acknowledgements**

We acknowledge Federal University of Pará (PROPESP and Fadesp) for technical support and National Counsel of Technological and Scientific Development and Coordination for Enhancement of Higher Education Personnel for fellowship support.

**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

**References**

1. Erstad DJ, Fuchs BC, Tanabe KK. Molecular signatures in hepatocellular carcinoma: A step toward rationally designed cancer therapy. Cancer 2018; 124:3084-104.
2. Zhang Z, Zhang G, Gao Z, et al. Comprehensive analysis of differentially expressed genes associated with PLK1 in bladder cancer. BMC Cancer 2017; 17:861.
3. Yin JG, Liu XY, Wang B, et al. Gene expression profiling analysis of ovarian cancer. Oncol Lett 2016;12:405-12.
4. Jiang HB, Yang TJ, Lu P, et al. Gene expression profiling of gastric cancer. Eur Rev Med Pharmacol Sci 2014;18 2109:15.
5. Hu S, Yuan H, Li Z, et al. Transcriptional response profiles of paired tumor-normal samples offer novel perspectives in pan-cancer analysis. Oncotarget 2017;8:41334-47.
6. de Assumpção PP, Dos Santos SE, Dos Santos ÂK, et al. The adjacent to tumor sample trap. Gastric Cancer 2016;19:1024-5.
7. Huang X, Stern DF, Zhao H. Transcriptional profiles from paired normal samples offer complementary information on cancer patient survival--evidence from TCGA pan-cancer data. Sci Rep 2016;6:20567.
8. Curtius K, Wright NA, Graham TA. An evolutionary perspective on field cancerization. Nat Rev Cancer 2018;18:19-32.
9. Virgilio E, Giarnieri E, Giovangoli MR, et al. Gastric juice microRNAs as potential biomarkers for screening gastric cancer: A systematic review. Anticancer Res 2018;38:613-6.
10. Abbas M, Habib M, Naveed M, et al. The relevance of gastric cancer biomarkers in prognosis and pre- and post- chemotherapy in clinical practice. Biomed Pharmacother 2017;95:1082-90.
11. Carломagno N, Incollingo P, Tammaro V, et al. Diagnostic, predictive, prognostic, and therapeutic molecular biomarkers in third millennium: A breakthrough in gastric cancer. Biomed Res Int 2017;2017:7869802.
12. Jin Z, Jiang W, Wang L. Biomarkers for gastric cancer: Progression in early diagnosis and prognosis (Review). Oncol Lett 2015;9:1502-8.
13. Thompson KJ, Ingle JN, Tang X, et al. A comprehensive analysis of breast cancer microbiota and host gene expression. PLoS One 2017;12:e0188873.
14. Garattini SK, Basile D, Cattaneo M, et al. Molecular classifications of gastric cancers: Novel insights and possible future applications. World J Gastrointest Oncol 2017;9:194-208.
15. Chen L, Zhu Z, Gao W, et al. Systemic analysis of different colorectal cancer cell lines and TCGA datasets identified IGF-1R/EGFR-PPAR-CASPASE axis as important indicator for radiotherapy sensitivity. Gene 2017;627:484-90.
16. Chang JT, Lee YM, Huang RS. The impact of the Cancer Genome Atlas on lung cancer. Transl Res 2015;166:568-85.
17. Zhang W. TCGA divides gastric cancer into four
molecular subtypes: implications for individualized therapeutics. Chin J Cancer 2014;33:469-70.
18. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. Cancer 1953;6:963-8.
19. Jakubek Y, Lang W, Vattathil S, et al. Genomic landscape established by allelic imbalance in the cancerization field of a normal appearing airway. Cancer Res 2016;76:3676-83.
20. Assumpçáo MB, Moreira FC, Hamoy IG, et al. High-throughput miRNA sequencing reveals a field effect in gastric cancer and suggests an epigenetic network mechanism. Bioinform Biol Insights 2015;9:111-7.
21. Dakubo GD, Jakupciak JP, Birch-Machin MA, et al. Clinical implications and utility of field cancerization. Cancer Cell Int 2007;7:2.
22. Baba Y, Ishimoto T, Kurashige J, et al. Epigenetic field cancerization in gastrointestinal cancers. Cancer Lett 2016;375:360-6.
23. Spitzwieser M, Holzweber E, Pfeiler G, et al. Applicability of HIN-1, MGMT and RASSF1A promoter methylation as biomarkers for detecting field cancerization in breast cancer. Breast Cancer Res 2015;17:125.
24. Takeshima H, Niwa T, Takahashi T, et al. Frequent involvement of chromatin remodeler alterations in gastric field cancerization. Cancer Lett 2015;357:328-38.
25. Trujillo KA, Jones AC, Griffith JK, et al. Markers of field cancerization: proposed clinical applications in prostate biopsies. Prostate Cancer 2012;2012:302894.
26. Luo Y, Yu M, Grady WM. Field cancerization in the colon: a role for aberrant DNA methylation? Gastroenterol Rep (Oxf) 2014;2:16-20.
27. Rivenbark AG, Coleman WB. Field cancerization in mammary carcinogenesis — Implications for prevention and treatment of breast cancer. Exp Mol Pathol 2012;93:391-8.
28. Vogelstein B, Kinzler KW. The Path to cancer — three strikes and you’re out. N Engl J Med 2015;373:1895-8.
29. Barra WF, Araújo TMT, Calcagno DQ, et al. Symptoms based cancer diagnosis — An inconceivable strategy. Cancer Re Rev 2017;1:1-3.
30. Schiffman JD, Fisher PG, Gibbs P. Early detection of cancer: past, present, and future. Am Soc Clin Oncol Educ Book 2015:57-65.
31. Wardle J, Robb K, Vernon S, et al. Screening for prevention and early diagnosis of cancer. Am Psychol 2015;70:119-33.
32. Kikuyama M, Kamisawa T, Kuruma S, et al. Early diagnosis to improve the poor prognosis of pancreatic cancer. Cancers (Basel) 2018;10:pii:E48.
33. Tang Y, Qiao G, Xu E, et al. Biomarkers for early diagnosis, prognosis, prediction, and recurrence monitoring of non-small cell lung cancer. Onco Targets Ther 2017;10:4527-34.
34. Hiom SC. Diagnosing cancer earlier: reviewing the evidence for improving cancer survival. Br J Cancer 2015;112 Suppl 1:S1-5.
35. Nakashima T, Tomita H, Hirata A, et al. Promotion of cell proliferation by the proto-oncogene DEK enhances oral squamous cell carcinogenesis through field cancerization. Cancer Med 2017;6:2424-39.
36. Patel A, Tripathi G, Gopalakrishnan K, et al. Field cancerisation in colorectal cancer: a new frontier or pastures past? World J Gastroenterol 2015;21:3763-72.
37. Bozic I, Antal T, Ohtsuki H, et al. Accumulation of driver and passenger mutations during tumor progression. Proc Natl Acad Sci U S A 2010;107:18545-50.