Modeling tumor development and metastasis using paired organoids derived from patients with colorectal cancer liver metastases

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Abstract

Tumor metastasis accounts for the majority of cancer-related deaths; it is therefore important to develop preclinical models that faithfully recapitulate disease progression. Here, we generated paired organoids derived from primary tumors and matched liver metastases in the same colorectal cancer (CRC) patients. Despite the fact that paired organoids exhibit comparable gene expression and cell morphology, organoids from metastatic lesions demonstrate more aggressive phenotypes, tumorigenesis, and metastatic capacity than those from primary lesions. Transcriptional analyses of the paired organoids reveal signature genes and pathways altered during the progression of CRC, including SOX2. Further study shows that inducible knockdown of SOX2 attenuated invasion, proliferation, and liver metastasis outgrowth. Taken together, we use patient-derived paired primary and metastatic cancer organoids to model CRC metastasis and illustrate that SOX2 is associated with CRC progression and may serve as a potential prognostic biomarker and therapeutic target of CRC.

Keywords: Colorectal cancer, Tumor metastasis, Preclinical model, Paired organoids, SOX2

To the Editor,

Tumor heterogeneity plays a key role in cancer progression and therapy resistance [1]. However, knowledge of how tumor heterogeneity arises and contributes to disease progression is still limited [2]. Recent advances in organoid culture have been successfully established in a variety of solid tumors [3–5].

Tumor organoids retain the histological complexity and genetic heterogeneity of parental tumors, even after many passages [6], providing a wide range of applications for cancer research. Organoids have enormous potential for the identification of optimal treatment strategies in individual patients [6]. For example, human CRC organoids derived from primary tumors [5] and liver metastases [7] have been reported as precision medical models for assessing drug responses. However, paired organoids have not been studied as a model for CRC progression. In the present study, we used paired organoids derived from primary and liver metastatic tumors of CRC patients to model cancer metastasis. Through in vitro and in vivo studies and transcriptional analyses of the paired organoids, we revealed key genes associated...
Fig. 1 (See legend on next page.)
with CRC liver metastasis, which could be translated into therapeutic targets or prognostic biomarkers for disease treatment. A total of 24 organoids have been established (Table S1). The library contained 2-paired organoid lines from patients P13 and P21. Particularly, P13 carried two primary tumors. The 13a and 13b organoids were established from the primary tumor, while 13L organoid was established from a synchronous liver metastatic tumor. Organoids of 21a and 21L were established from primary tumor and synchronous liver metastasis of the patient P21, which data demonstrated in Additional files (Supplementary Table S1 and Fig. S1, S2, S4, and S5). Histopathological structures and the intestinal epithelial marker CDX2 of parental tumor were well preserved in organoids (Fig. S1).

Invasion is a fundamental step in tumor progression toward metastasis. To study collective invasion, we cultured paired organoids in a 3D invasion matrix (Fig. 1a). Although we did not observe collective protrusive migration in organoids derived from primary lesions, metastatic organoids exhibited robust protrusive migration into 3D invasion matrix (Fig. 1b and Fig. S2A and B). Besides, the expression level of MMP-2 (matrix metalloproteinase 2) and Ki67 was significantly higher in metastatic organoids than that in the primary organoids (Fig. S2C-E). In subcutaneous xenotransplantation of paired organoids (Fig. 1c), the growth rate and volume of 13L organoids derived xenograft tumors was significantly higher than that of 13a and 13b organoids derived tumors (Fig. 1d and e). Furthermore, we successfully generated organoids from xenografts, histology, and Ki-67 expression analysis of xenografts, as well as organoids derived from these xenografts, demonstrated similarity to the original parental tumors (Fig. 1f and Fig. S2F). We next performed splenic injection of the paired organoids to assess the development of liver metastases (Fig. 1g). The 13L organoids formed macrometastatic tumors in the livers (Fig. 1h and i), whereas 13b organoids and 13a organoids failed to colonize and had a negative expression of Ki67 in the liver (Fig. S2G).

We then performed gene expression analysis in the paired organoids and tumor tissue from patient 13. There were 33 genes ($P < 0.05$; fold change > 2.5) that were significantly upregulated in metastatic organoids (Fig. 2a and Fig. S3A and B), including the transcription factor SOX2. Previous studies have shown that SOX2 plays critical roles in embryonic pluripotent stem cells [8] and that SOX2 is abnormally expressed in many types of cancer [9–12]. The differential expression of SOX2 in paired organoids was consistent with the RNA-seq data (Fig. 2b and c), and SOX2 was also highly expressed in the metastatic tissues (Fig. 2d), while relatively low expression in normal colon tissues (Fig. S3C-F). SOX2 is also highly expressed in metastatic organoids and tissues of the other paired organoids (Fig. S4A and B).

To investigate the role of SOX2 in CRC progression, doxycycline (Dox) inducible expression of shRNA targeting SOX2 was established in metastatic organoids (Fig. S4C and D). SOX2+ organoids exhibited the reduced ability of invasion, colony-forming efficiency, and cell viability in metastatic organoid lines (Fig. 2e-g and Fig. S4E-G). Furthermore, the metastatic organoids efficiently formed large metastatic tumors in control groups (Dox untreated), whereas the SOX2− organoids showed no or few engraftments (Fig. 2h). The downregulation of SOX2 and Ki67 was further confirmed by immunohistochemistry (Fig. 2i and Fig. S4H). We then overexpressed SOX2 in primary organoids and found that organoids with overexpressed SOX2 exhibited increased ability of invasion and proliferation when compared with control organoids (Fig. S5). Taken together, these findings demonstrate that SOX2 expression is sufficient and necessary for CRC organoids to exhibit the metastatic potential.
Fig. 2 (See legend on next page.)
In summary, the present study highlights the potential of patient-derived paired primary and metastatic cancer organoids as an experimental model for investigating CRC progression. We identified a significantly dysregulated gene between paired organoids, SOX2, which could be a prognostic biomarker, and perhaps a potent therapeutic target in the treatment of CRC.

Supplementary information

Additional file 1: Supplementary Table S1. Summary of patient-derived CRC organoid lines and corresponding clinical data.

Additional file 2: Supplementary Figures. Figure S1. Paired organoids derived from primary and metastatic CRC recapitulate the histopathological structure of parental tumor. A and B, Organoids architecture resembles parental tumor epithelium. Representative bright-field images of organoids together with H&E staining of parental tumors and patient-derived organoids. The scale bar represents 100 μm. C and D, Representative IHC sections for the intestinal epithelial marker CDX2. The scale bar represents 100 μm. Figure S2. Organoids derived from liver metastatic lesions exhibited the most aggressive phenotypes with significant high tumorigenic and metastatic potential. A, Representative micrographs of organoids in 3D invasion assay. Tumor organoids showed the smooth and protrusive leading fronts, respectively. B, Micrographs of the paired organoids stained with phalloidin-F-actin (right). The scale bar represents 100 μm. C, qRT-PCR analysis of MMP-2 in paired organoid lines. Error bars indicate SEMs. *P = 0.0027, **P = 0.0083, ***P = 0.0006 (one-way ANOVA; left), ***P = 0.0406 (Unpaired t test; right). D, Western blot analysis of the MMP-2 protein expression in paired organoid lines. α-tubulin was used as a loading control. E, Representative IHC sections for K667 in human colorectal tumor tissues and paired organoid lines. The scale bar represents 100 μm. F, Representative IHC sections for K667 in organoid xenografts and organoids derived from xenografts. The scale bar represents 100 μm. G, Representative gross image, histopathology and K667 staining of whole liver from primary organoid xenografts. The scale bar of the whole liver represents 1 cm. H, Representative micrographs of colonies arising from the control, LV-Vector and LV-SOX2 primary organoid lines. The scale bar represents 200 μm. I, Representative micrographs of colonies arising from the 21L-shRNA-1/2 organoids (left) and ratio of Ki67-positive tumor cells in liver metastases (right). Scale bars, 200 μm (top) and 100 μm (bottom). J, Representative micrographs of colonies arising from the 21L-shRNA-1/2 organoids (left) and ratio of Ki67-positive tumor cells in liver metastases (right). Scale bars, 200 μm (top) and 100 μm (bottom). K, Representative micrographs of the 13L-shRNA-1/2 organoids stained with phalloidin-F-actin. The scale bar represents 200 μm. L, Representative micrographs of colonies arising from the 13L-shRNA-1/2 organoids (left) and ratio of Ki67-positive tumor cells in liver metastases (right). Scale bars, 200 μm (top) and 100 μm (bottom).

Additional file 3. Supplementary Materials and Methods.

Abbreviations
CRC: Colorectal cancer; 3D: Three-dimensional; MMP-2: Matrix metalloproteinase 2; qRT-PCR: Quantitative reverse transcription PCR; Dox: Doxycycline; CTG: CellTiter-Glo Luminescent
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Authors’ contributions
Conception and design: MH, HL, JZ, and GC; development of methodology: HL, WD, RW, XX, and JZ; acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): HL, JZ, LH, RW, SM, WX, and LD; analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): HL, WD, RW, XX, JZ, GX, JY, and JZ; manuscript writing: HL; manuscript revision: HL, XX, MH, NL, JZ, and GC; administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): MH, HL, JZ, and GC; study supervision: JZ and GC. All authors read and approved the final manuscript.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study was approved by the Shanghai Cancer Center of Fudan University Ethics Committee. Written informed consent was obtained prior to the acquisition of tissue from all patients. All animal procedures were performed under guidelines approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009;458(7239):719–24.
2. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. Cell. 2017;168(4):613–28.
3. Broust L, Mastrogiovanni G, Veersteegen MM, Francis HE, Gavrilov LM, Bradshaw CR, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med. 2017;23(12):1424–35.
4. Sachs N, de Ligt J, Kopper D, Gogola E, Bounova G, Weeber F, et al. A living biobank of breast cancer organoids captures disease heterogeneity. Cell. 2018;172(1-2):373–86 e10.
5. van de Wetering M, Francis HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell. 2015;161(4):933–45.
6. Yoshida GJ. Applications of patient-derived tumor xenograft models and tumor organoids. J Hematol Oncol. 2020;13(1):4.
7. Vlachogiannis G, Hedaya Y, Savidis A, Jamin Y, Fernandez-Mateos J, Khan K, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 2018;359:920–6.
8. Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 2003;17(11):126–40.
9. Leis O, Eguia A, Lopez-Aribillaga E, Alberdi MI, Hernandez-Garcia S, Brion A, et al. Sox2 expression in breast tumours and activation in breast cancer stem cells. Oncogene. 2012;31(11):1354–65.
10. Zhou YT, Lee CC, Hsiao SH, Lin SE, Lin SC, Chung CH, et al. The emerging role of SOX2 in cell proliferation and survival and its crosstalk with oncogenic signaling in lung cancer. Stem Cells. 2013;31(12):2607–19.
11. Lundberg IV, Lofgren Biström A, Edin S, Eldof V, Oberg A, Sterling R, et al. SOX2 expression is regulated by BRAF and contributes to poor patient prognosis in colorectal cancer. PLoS One. 2014;9(7):e101957.
12. Neumann J, Bahm F, Horst D, Kriegel L, Engel J, Luque RM, et al. SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer. BMC Cancer. 2011;11:518.

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