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Editorial

Multiplex immunohistochemistry of metastatic colorectal cancer and *ex vivo* tumor avatars

Patient-derived tumor organoids are three-dimensional *ex vivo* models of the neoplastic cell population of human solid tumors [1]. Cultured tumor cells self-organize into organoid structures when supplied with suitable media and niche factors in extracellular matrix scaffolds [2,3]. Tumor organoids recapitulate the genomic makeup [4,5] and several pathophysiological traits of tumors [2], and are increasingly implemented as a powerful tool in translational cancer research [3,6,7].

The journal cover image shows a "yin and yang" representation of tumor tissue and cultured tumor organoids from a patient with rectal cancer and metastasis to the liver (the samples were taken from two distinct hepatic metastases). Both samples are stained with a multiplex of diagnostic protein markers of colorectal cancer [8,9], and indicate complementarity of the *in situ* and *ex vivo* sample counterparts (Fig. 1). Both demonstrate a well-differentiated phenotype with cystic structures of epithelial cell layers and simple columnar cells. The elongated basal localization of the nuclei and cytokeratin 20 in the luminal sides of selected structures further indicate cell polarity and molecular tumor heterogeneity in both samples. Only the tumor tissue showed a non-neoplastic tumor stroma, indicated by cells positive for cytokeratin 7 and negative for caudal type homeobox 2, and consistent with the exclusive propagation of malignant epithelial cells in organoids.

The "yin and yang" complementarity of tumor tissue samples and organoids presents opportunity for a next generation of precision cancer medicine. Combined tumor biomarker analysis and *ex vivo* drug sensitivity testing can model the activity of cancer therapies in a personalized manner. Observational "co-clinical" analyses have shown that tumor organoids can model clinical treatment responses in metastatic colorectal cancer, including to targeted anti-EGFR therapy [10] and to chemotherapies with irinotecan [7]. However, no published prospective intervention studies have yet confirmed the power of organoids to guide the selection of effective experimental therapies for patients [11]. The journal cover image was captured from an *ex vivo* pharmacogenomics study of patients treated by hepatic resection for metastatic colorectal cancer. The living biobank includes separate PDO lineages of multiple liver lesions from each patient, allowing drug sensitivity testing in the context of metastatic tumor heterogeneity [3,12]. A large *ex vivo* pharmacogenomics resource has been established in the pre-clinical phase of the study (partly unpublished data), and this will serve as a reference dataset for prospective nomination of standard and experimental anti-cancer agents in a planned and approved single-arm intervention study for treatment of patients with metastatic colorectal cancer (EU Clinical Trials Register number: 2020–003395-41).

Methods

Liver metastases from a patient treated by hepatic resection for metastatic rectal cancer at Oslo University Hospital were obtained after written informed consent, in accordance with the Declaration of Helsinki, the Norwegian Data Protection Authority and the Regional Committee for Medical and Health Research Ethics, South Eastern Norway (reference: 2010/1805 and 2017/780). Tumor organoids were grown as previously described [12]. Both the tumor tissue and the organoids were formalin-fixed, paraffin-embedded and sectioned as 3 μm slices and stained by multiplex fluorescence-based immunohistochemistry using Opal kits and reagents (Akoya Biosciences) [12]. Antibodies and fluorophores used were: anti-CDX2 (1:400, clone EPR2764Y, Cell Marque) detected by Opal 570, anti-CK20 (1:1000, clone Ks20.8, Agilent Dako) detected by Opal 520, anti-CK7 (1:400, clone OV-TL 12/30, Agilent Dako) detected by Opal 620, anti-ECAD (1:10.000, clone 36, BD Biosciences) detected by Opal 690. Cell nuclei were stained with DAPI. Multispectral images were acquired with the Vectra3 imaging system and unmixed in inForm Software (Akoya Biosciences) [13].

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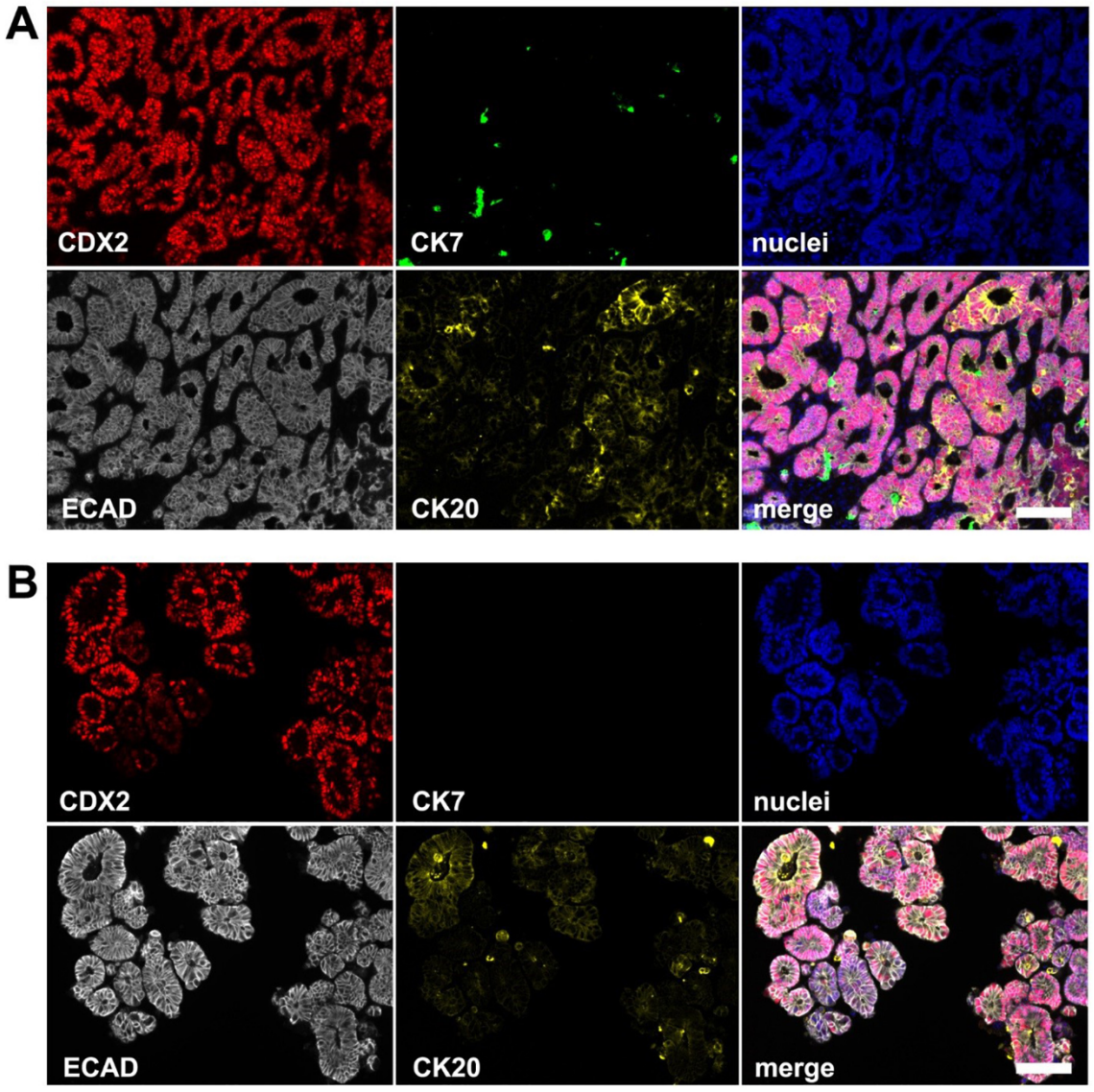

Fig. 1. Multiplex immunohistochemistry of colorectal liver metastasis and tumor organoids.
Multiplex staining of four protein markers in A) a liver metastasis tissue sample and B) tumor organoids of a separate lesion from a patient diagnosed with metastatic rectal cancer. The merged images in the bottom right panels are shown in the "yin and yang" representation in the cover image. Red: caudal type homeobox 2 (CDX2); green: cytokeratin 7 (CK7); blue: cell nuclei (DAPI); white: e-cadherin (ECAD); yellow: cytokeratin 20 (CK20). Scale bar: 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Declaration of Competing Interest

The authors declare no competing financial interests.

References

[1] J. Drost, H. Clevers, Organoids in cancer research, Nat. Rev. Cancer 18 (2018) 407–418.

[2] M. Fujii, M. Shimokawa, S. Date, A. Takano, M. Matano, K. Nanki, Y. Ohta, K. Toshimitsu, Y. Nakazato, K. Kawasaki, et al., A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis, Cell Stem Cell 18 (2016) 827–838.

[3] J. Bruun, K. Kryeziu, P.W. Eide, S.H. Moosavi, I.A. Eilertsen, J. Langerud, B. Rosok, M.Z. Totland, T.H. Brunsell, T. Pellinen, et al., Patient-derived organoids from multiple colorectal cancer liver metastases reveal moderate intra-patient pharmacotranscriptomic heterogeneity, Clin. Cancer Res. 26 (2020) 4107–4119.

[4] F. Weeber, M. van de Wetering, M. Hoogstraat, K.K. Dijkstra, O. Krijgsman, T. Kuilman, C.G. Gadellaa-van Hooijdonk, D.L. van der Velden, D.S. Peeper, E. P. Cuppen, et al., Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 13308–13311.

[5] M. van de Wetering, H.E. Francies, J.M. Francis, G. Bounova, F. Iorio, A. Pronk, W. van Houdt, J. van Gorp, A. Taylor-Weiner, L. Kester, et al., Prospective derivation of a living organoid biobank of colorectal cancer patients, Cell 161 (2015) 933–945.

[6] G. Vlachogiannis, S. Hedayat, A. Vatsiou, Y. Jamin, J. Fernandez-Mateos, K. Khan, A. Lampis, K. Eason, I. Huntingford, R. Burke, et al., Patient-derived organoids model treatment response of metastatic gastrointestinal cancers, Science 359 (2018) 920–926.

[7] S.N. Ooft, F. Weeber, K.K. Dijkstra, C.M. McLean, S. Kaing, E. van Werkhoven, L. Schipper, L. Hoes, D.J. Vis, J. van de Haar, et al., Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients, Sci. Transl. Med. 11 (2019).

[8] A. Lugli, A. Tzankov, I. Zlobec, L.M. Terracciano, Differential diagnostic and functional role of the multi-marker phenotype CDX2/CK20/CK7 in colorectal cancer stratified by mismatch repair status, Mod. Pathol. 21 (2008) 1403–1412.

[9] J. Bruun, A. Sveen, R. Barros, P.W. Eide, I. Eilertsen, M. Kolberg, T. Pellinen, L. David, A. Svindland, O. Kallioniemi, et al., Prognostic, predictive, and pharmacogenomic assessments of CDX2 refine stratification of colorectal cancer, Mol. Oncol. 12 (2018) 1639–1655.

[10] M. Schutte, T. Risch, N. Abdavi-Azar, K. Boehnke, D. Schumacher, M. Keil, R. Yildiriman, C. Jandrasits, T. Borodina, V. Amstislavskiy, et al., Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors, Nat. Commun. 8 (2017) 14262.

[11] S.N. Ooft, F. Weeber, L. Schipper, K.K. Dijkstra, C.M. McLean, S. Kaing, J. van de Haar, W. Prevoo, E. van Werkhoven, P. Snaebjornsson, et al., Prospective experimental treatment of colorectal cancer patients based on organoid drug responses, ESMO Open 6 (2021), 100103.

[12] K. Kryeziu, S.H. Moosavi, C.H. Bergsland, M.G. Guren, P.W. Eide, M.Z. Totland, K. Lassen, A. Abildgaard, A. Nesbakken, A. Sveen, R.A. Lothe, Increased sensitivity to SMAC mimetic LCL161 identified by longitudinal ex vivo pharmacogenomics of recurrent, KRAS mutated rectal cancer liver metastases, J. Transl. Med. 19 (2021) 384.

[13] N. Lopes, C.H. Bergsland, M. Bjornslett, T. Pellinen, A. Svindland, A. Nesbakken, R. Almeida, R.A. Lothe, L. David, J. Bruun, Digital image analysis of multiplex fluorescence IHC in colorectal cancer recognizes the prognostic value of CDX2 and its negative correlation with SOX2, Lab. Invest. 100 (2020) 120–134.

Kushtrim Kryeziu[a], Christian H. Bergsland[a,b], Tormod K. Guren[c], Anita Sveen[a,b], Ragnhild A. Lothe[a,b,*]

[a] *Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway*

[b] *Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway*

[c] *Department of Oncology, Oslo University Hospital, Oslo, Norway*

[*] Corresponding author at: Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway.
E-mail address: ragnhild.a.lothe@rr-research.no (R.A. Lothe).