Organoid culture of bile from advanced cancer patients and its clinical application

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Majority of the patients with biliary tract cancer (BTC) are found inoperable at the initial diagnosis. Consequently, they are often treated by conventional chemotherapy, but BTCs are generally not sensitive to cytotoxic agents, leading to the poor prognosis requiring future development of more effective, molecular-targeted therapeutics. Organoid culture is an emerging technique that enables long-term propagation of primary cells in a physiological setting. This technique has been successfully applied to BTC specimens as well, if successfully resected by surgery. Samples from patients with more advanced BTC, however, have not been subject to organoid culture, partly because collection of tumors in an invasive way, such as percutaneous ultrasound-guided biopsy, could be hardly justified without therapeutic purposes. On the other hand, bile from advanced cancer patients could be alternatively obtained in a relatively non-invasive manner during therapeutic endoscopic retrograde cholangiopancreatography (ERCP), which is conducted to relieve obstructive jaundice caused by disease progression. However, collected samples are frequently accompanied by severe bacterial infection, making it difficult to think of bile as a starting material for cell culture.

As we knew that bile collected during ERCP treatment for cytology examination frequently contained cancer cells, we asked if we could propagate those cancer cells as organoids. By optimizing various processes including vigorous washing and prompt sample processing, we finally established a robust way for organoid culture of bile-derived cells. A total of 82 patients with biliary disease who underwent ERCP have been subject to bile sampling for 3D culture. Organoids were characterized in various aspects, including histology, immunochemistry and tumorigenecity in immunodeficient mice. Success rate for organoid culture from bile was as high as 89% (73/82cases). Organoids were also obtained from surgically resected BTCs and from bile of non-cancer patients with biliary tract stenosis due to stones. Histological studies revealed atypical cribriform structures in tumor-derived organoids, but simple cystic structure in organoids derived from bile of non-malignant patients. Immunopositivity for p53, suggestive of its mutation, was observed in 32%, and HER2 positivity in 20% of the cases tested. About 45 % of organoids developed subcutaneous tumors in nude mice.

Collectively, we successfully conducted organoid culture of advanced biliary tumors from bile collected by ERCP. Recovered organoids well reflected the nature of underlying diseases, potentially contributing to development of companion diagnosis and novel therapeutics for BTC.

Honors

2005 Research Fellowship, The Uehara Memorial Foundation
2013 Young Investigator Award, Pancreas Research Foundation of Japan
2013 Research Grants, Princess Takamatsu Cancer Research Fund
2014 Relay-for-Life Research Grants, Japan Cancer Society
2015 Research Grants, The Naito Foundation

Publications

1. Sato T, Morita M, Tanaka R, Inoue Y, Nomura M, Sakamoto Y, Miura K, Ito S, Sato I, Tanaka N, Abe, Takahashi S, Kawai M, Sato M, **Hippo Y**, Shima H, Okada Y and Tanuma N. Ex vivo model of non-small cell lung cancer using mouse lung epithelial cells. ***Oncology Lett***. 14: 6863-6868, 2017
2. Maru Y, Orihashi K, **Hippo Y**. Lentivirus-based stable gene delivery into intestinal organoids. ***Methods Mol Biol***, 1422:13-21. 2016
3. Ochiai M, **Hippo Y***, Izumiya M, Watanabe M, Nakagama H. Newly Defined Aberrant Crypt Foci as a Marker for Dysplasia in the Rat Colon. ***Cancer Sci.*** 105(8):943-50. 2014 (* corresponding author)
4. Onuma K, Ochiai M, Orihashi K, Takahashi M, Imai T, Nakagama H, **Hippo Y**. Genetic reconstitution of tumorigenesis in primary intestinal cells. ***Proc Natl Acad Sci U S A.*** 110(27):11127-32. 2013

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