DISCUSSION

We describe histological and electron-microscopical characteristics of lymphatic invasion of the bile duct cancer using 3D human stromal tissue model. TFK-1 human bile duct cancer cells morphologically became spindle in shape, i.e., EMT when they entered the lymphatic vessels. The TFK-1 cells formed spherical nests in the lymphatic vessels.

The most cases of extrahepatic bile duct cancer are epithelial tumors that originate from the bile ducts, and represent one of the highly aggressive malignancies [7-10]. In a recent study, the median disease-specific survival rate after surgery in patients with lymph node metastasis was lower than that of patients without lymph node metastasis [11]. It is an important issue to analyze the mechanisms of the lymphatic invasion leading to lymph node metastasis. However, molecular/histological mechanisms of lymphatic invasion have not yet clarified, while the metastatic cascade via blood vessel have been demonstrated recently.

Malignancy in cancers is characterized by invasion and metastasis that are closely associated with interaction between cancer cells and non-cancerous stroma [12,13]. Recent studies have demonstrated invasion-metastasis cascade such as invasion of basement membrane, passage through extracellular matrix, intravasation, vascular dissemination, extravasation, and formation of metastatic foci [14,15]. However, these sequential steps of the cascade are associated with blood vessels, but not lymphatic vessels. Mechanisms of lymphatic spread have not yet understood. Our study is the first to demonstrate histological/electron-microscopical findings of lymphatic invasion using human extrahepatic bile duct cancer cells.

EMT is an important process that occurs during the cancer progression. It is characterized by the loss of epithelial factors, including E-cadherin, claudins, and cytokeratin's, and the upregulated expression of mesenchymal markers, including N-cadherin, vimentin and fibronectin. Cancer cells that have undergone EMT are thought to acquire a fibroblast-like motile and an invasive phenotype [16-19]. TFK-1 human bile duct cancer cells morphologically showed EMT at the start of stromal invasion. Moreover, TFK-1 cells significantly exhibited EMT at the lymphatic intravasation/extravasation, and formed spherical nests in the lymphatic vessels. These processes are extremely consistent with the invasion-metastasis cascade of blood vessel.

In conclusion, we demonstrate morphological characteristics of lymphatic invasion of the extrahepatic bile duct cancer, using 3D human stromal tissues with lymphatic vessels. The 3D tissues with lymphatic vessels are thought to become a useful model analyzing the lymphatic invasion mechanisms.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

1. Kijima H, Wu Y, Yoshizawa T, Suzuki T, Tsugeno Y, et al. (2014) Pathological characteristics of early to advanced gallbladder carcinoma and extrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci 21: 453-458.

2. Albores-Saavedra J, Henson DE, Klimstra DS (2015) Tumors of the gallbladder, extrahepatic bile ducts and ampulla of Vater. Atlas of tumor pathology. 4th series, fascicle 23. Armed Forces Institute of Pathology (AFIP), Washington DC. pp: 303-374.

3. WHO classification of tumors editorial board (2019) Digestive system tumor. WHO classification of tumors, 5th ed. Lyon: IARC Press. pp: 289-291.

4. Saijyo S, Kudo T, Suzuki M, Katayose Y, Shinoda M, et al. (1995) Establishment of a new extrahepatic bile duct carcinoma cell line, TFK-1. Tohoku J Exp Med 177: 61-71.

5. Nishiguchi A, Matsusaki M, Kano MR, Nishihara H, Okano D, et al. (2018) In vitro 3D blood/lymph-vascularized human stromal tissues for preclinical assays of cancer metastasis. Biomaterials. 179: 144-155.

6. Oikiri H, Asano Y, Matsusaki M, Akashi M, Shimoda H, et al. (2019) Inhibitory effect of carbonyl reductase 1 against peritoneal progression of ovarian cancer: evaluation by ex vivo 3D-human peritoneal model. Mol Biol Rep. 46: 4685-4697.

7. Yoshizawa T, Ishido K, Saito K, Haga T, Seino H, et al. (2017) Prognostic impact of extracapsular lymph node invasion and myofibroblastic activity in extrahepatic bile duct cancer. Clin Med Insights Pathol 10: 1179555717729652.

8. Yoshizawa T, Toyoki Y, Hirai H, Haga T, Toba T, et al. (2014) Invasive micropapillary carcinoma of the extrahepatic bile duct and its malignant potential. Oncol Rep 32: 1355-1361.

9. Ohashi M, Kusumi T, Sato F, Kudo Y, Jin H, et al. (2009) Expression of syndecan-1 and E-cadherin is inversely correlated with poor patient's prognosis and recurrent status of extrahepatic bile duct carcinoma. Biomed Res 30: 79-86.

10. Okano K, Yoshizawa T, Miura T, Ishido K, Kudo D, et al. (2018) Impact of the histological phenotype of extrahepatic bile duct carcinoma. Mol Clin Oncol 8: 54-60.

11. Ito K, Ito H, Allen PJ, Gonen M, Klimstra D, et al. (2010) Adequate lymph node assessment for extrahepatic bile duct adenocarcinoma. Ann Surg 251: 675-681.

12. Miura T, Yoshizawa T, Hirai H, Seino H, Morohashi S, et al. (2017) Prognostic Impact of CD163+ Macrophages in Tumor Stroma and CD8+ T-Cells in Cancer Cell Nests in Invasive Extrahepatic Bile Duct Cancer. Anticancer Res 37: 183-190.

13. Goto S, Ishido K, Yoshizawa T, Haga T, Morohashi S, et al. (2019) Histopathological Characteristics of Pancreatic Cancer Stroma Induced by Neoadjuvant Chemotherapy. Bomed Res J 3: 61-64.

14. Weinberg RA (2013) The Biology of Cancer, 2nd ed. New York, NY: Garland Science. pp: 643-651.

15. Kumar V, Abbas AK, Aster JC (2015) Robbins and Cotran Pathologic Basis of Disease, 9th ed. Philadelphia, PA: Elsevier Saunders. pp: 306-310.

16. Thiery JP, Sleeman JP (2006) Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7: 131-142.

17. Jing Yang J, Weinberg RA (2008) Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14: 818-829.

18. Wu Y, Sato F, Yamada T, Bhawal UK, Kawamoto T, et al. (2012) The BHLH transcription factor DEC1 plays an important role in the epithelial-mesenchymal transition of pancreatic cancer. Int J Oncol 41: 1337-1346.

19. Tsugeno Y, Yoshizawa T, Wu Y, Goto S, Haga T, et al. (2019) Type I Collagen Induces DEC Expression and Epithelial-Mesenchymal Transition (EMT) in Human Breast Cancer MCF-7 Cells. BioMed Res J 3: 125-131.