Editorial

Potential of extracellular vesicles and exosomes as diagnostic markers for cholangiocarcinoma

Linping Cao[1], Jiawei Hong[2], Jian Wu[1]

[1]Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China; [2]Key Lab of Combined Multi-Organ Transplantation, Ministry of Public Health, Hangzhou, China

Correspondence to: Jian Wu. Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, First Affiliated Hospital, School of Medicine, Zhejiang University, 79# Qingchun Road, Hangzhou 310003, China. Email: drwujian@zju.edu.cn.

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Cholangiocarcinoma (CCA), a malignant carcinoma originating from biliary epithelium, is the second largest primary liver malignancy, accounting for 10–15% of hepatobiliary malignancies. Macroscopically, CCA is a fatal malignancy, with a 5-year survival rate of less than 10% (1). The lack of typical symptoms and signs makes early CCA difficult to identify and diagnose by early health screening. The diagnosis of CCA mainly relies on radiography, tumor biomarkers, invasive diagnosis, as well as pathological examination, which results in the majority of patients being detected in the middle and late stages, and only about one-third of CCA patients have access to treatment (1).

The development of CCA has a number of triggers, and is associated with multiple known factors (such as biliary calculi, biliary inflammation, primary sclerosing cholangitis, parasitic infections, toxins, and liver viruses) and some potential risk factors (such as diabetes, obesity, and alcohol) (1). However, establishing a non-invasive diagnostic strategy for CCA remains challenging, and there is currently a lack of accurate biomarkers (2). Most CCAs are sporadic, resulting in difficulties in terms of epidemiological surveillance of the disease. In addition, the commonly used biomarker of CCA, carbohydrate antigen 19-9 (CA19-9), lacks diagnostic sensitivity and specificity, and can also be affected by bacterial cholangitis, cholestasis, and other factors (3). Therefore, identifying effective and appropriate tumor biomarkers for the early clinical diagnosis of CCA is crucial.

Extracellular vesicles (EVs) are membrane-coated spheres secreted by live or dying cells and are present in various biological fluids (4). Microscopically, EVs have unique and diverse biomolecular compositions (e.g., proteins, RNA, DNA, metabolites, and lipids) (4). CCA is typically characterized by the incorporation of cancer cells into a dense stroma containing fibroblasts, lymphocytes, and various immune cells. EVs can mediate the transfer of proteins and genes between different cells, leading to the overexpression of oncogenes in recipient CCA cells as well as drug-resistance to chemotherapy agents. At the same time, EVs mediate the transfer of information between multiple cells, influence the tumor cell microenvironment, and promote the development of CCA (5). It is also worth mentioning that most EVs detected in the serum of CCA patients are exosomes with a high abundance of carcinogenic proteins (6).

Exosomes are the major component of EVs. They are membrane structures (between 40 and 100 nanometers in length) that are secreted into body fluids by various living or dying cells, and contain multiple RNAs and proteins. Exosomes deliver nucleic acids or protein to the desired cells, and are considered to be significant mediators of information exchange and material transfer between different cells. Exosomes can be detected in the serum, urine, saliva, bile, ascites, amniotic fluid, milk, and other bodily fluids (7). Exosomes derived from tumor are highly associated with tumor progression, metastasis, invasion, and drug-resistance. Apoptotic exosomes from dying cells treated with chemotherapeutic agents can promote the drug-resistance of tumors in other locations that are not exposed to those chemotherapeutic agents. Exosomes have been used as diagnostic biomarkers in numerous malignant carcinomas. Thus, whether EVs and exosomes have

potential diagnostic and prognostic value in CCA is worth exploring.

At present, many scholars have confirmed the potential application value of exosomes as tumor markers in CCAs. CCAs have specific exosomal mRNAs, microRNAs, long non-coding RNAs (lncRNAs), exosomal piwi-interacting RNAs, (piRNAs) and circular RNAs (circRNAs) spectra, which play significant roles in tumor progression and are abundant in EVs or exosomes that have potential neoplastic diagnostic value (8-12). For instance, Ge *et al.* verified that with the advancement of cancer TNM stages of CCA, the level of lncRNAs detected in serum exosomes was significantly increased, suggesting that patients with a high lncRNA expression level had a poorer prognosis (10). Furthermore, Gu *et al.* reported that the level of piRNAs in the serum exosomes of CCA patients revealed a similar trend as lncRNAs (11), suggesting a worse prognosis in patients with a high level of piRNA transcription expression.

In addition, some specific proteins or compounds contained in EVs can also be used as CCA diagnostic markers. Ikeda *et al.* demonstrated that exosomal Claudin-3, which is a transmembrane protein that participates in epithelial tight junctions, in EVs could be a potential tumor biomarker (13). The abundance of multiple cancer-related microRNAs in EVs has a significant influence on the process of tumor malignancy, and some exosome contents can also be applied as prognostic indicators of CCA. For example, Shen *et al.*'s research showed that elevated levels of exosome miR-200c-3p as well as the exosomal miR-200 family can be used as independent risk factors for CCA patients' recurrence prediction, which will lead to stronger invasion and chemoresistance (9).

Moreover, tumorigenesis may also be affected by high or low gene expression in EVs and exosomes, which could provide a theoretical explanation for EVs and exosomes as diagnostic markers of CCA. The overexpression of certain oncosuppressor genes has been detected in exosomes; for instance, overexpression of the miR-30e family in the EVs of CCA cells inhibits cell invasion and migration by inhibiting the TGF-β-induced epithelial-mesenchymal transition (EMT) pathway (14). Accordingly, artificially inhibiting the miR-30e family in CCA cells can promote TGF-β-induced EMT, invasion, and migration.

Meanwhile, there are some down-regulated cancer-related RNAs in exosomes and EVs, which can also be reviewed as biomarkers. For example, the expression of the miR-34c family in exosomes is decreased, which mediates fibroblast activation by directly targeting the expression of WNT1 in exosomes. In addition, down-regulated activation of cancer-associated fibroblasts (CAFs) via increased secretion of IL-1β, IL-6, and IL-8, as well as exosome miR-34c, can lead to the promotion of CCA growth, invasion, and resistance (15).

In conclusion, correctly understanding the roles of EVs and exosomes in CCA is crucial for the clinical diagnosis and treatment of CCAs. Therefore, more and larger randomized controlled trials are required to evaluate the potential of EVs and exosomes as tumor biomarkers in CCA patients.

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