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Original contribution

Expression pattern of cancer-associated fibroblast and its clinical relevance in intrahepatic cholangiocarcinoma☆

Xiao-fang Zhang MD[a,b,1], Min Dong MD[b,c,1], Yu-hang Pan MD[a,b], Jian-ning Chen MD[a,b], Xiang-qi Huang MD[a,b], Yi Jin MD[a,b], Chun-kui Shao MD, PhD[a,b,*]

[a]*Department of Pathology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510630, China*
[b]*Guangdong Provincial Key Laboratory of Liver Disease Research, Guangzhou, China*
[c]*Department of Medical Oncology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510630, China*

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Summary Intrahepatic cholangiocarcinoma (ICC) is a highly malignant neoplasm and lack of effective treatment, characterized by dense desmoplastic stroma rich in cancer-associated fibroblasts (CAFs), which have been indicated to facilitate tumor progression in several types of human cancer. However, the clinical relevance of CAFs in ICC has not been fully characterized. Here, we evaluated the histological phenotype of CAFs and immunohistochemical expressions of α-SMA, FSP-1, and PDGFRβ in 71 ICC cases, and found that immature CAF phenotype was significantly associated with lymph node metastasis ($P = .045$), advanced TNM stage ($P = .025$) and poor 5-year overall survival (OS) (38.5% versus 78.6%, $P = .015$). In addition, α-SMA, FSP-1, and PDGFRβ were positively expressed in stromal fibroblasts in 63.4% (45/71), 84.5% (60/71), and 78.9% (56/71) of patients, respectively. Positive expression of α-SMA was correlated with poor differentiation ($P = .032$); FSP-1 expression in stromal fibroblasts was linked with lymph node metastasis ($P = .022$) and immature phenotype ($P = .048$). What's more, positive expression of FSP-1 in cancer cells was observed in 22.5% (16/71) of cases and was correlated with worse 5-year OS (36.4% versus 76.7%, $P = .014$). Importantly, in multivariate analysis, histological CAF phenotype was an independent prognostic factor for OS in ICC. Our findings demonstrated histological categorization of CAFs was a useful predictor for prognosis, providing new evidence that CAFs play a crucial role in tumor progression and can serve as potential therapeutic targets in ICC.

1. Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic tumor [1]. Although it has been viewed as a relatively rare malignancy, interest in this disease is rising as the incidence of ICC increased markedly worldwide and its mortality rate remains high, with a 5-year overall survival (OS) rate of less than 5% [2]. Lacking specific symptoms, the majority of patients with ICC are diagnosed at

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* Corresponding author: Department of Pathology, the Third Affiliated Hospital, Sun Yat-sen University, 600 Tianhe Rd, Guangzhou, 510630, China.
E-mail address: chunkuishao2011@163.com (C. Shao).

[1] Xiao-fang Zhang and Min Dong contributed equally to this work.

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advanced stages. Surgical resection offers the only strategy for curative treatment, but the recurrence and metastasis rates of advanced ICC were still high even after surgical resection; a previous study had reported a recurrence of more than 50% following resection of ICC [3]. Its resistance to chemo- and radio-treatment and lack of effective molecular targeted therapy also contribute to the poor outcome of patients [4]. Therefore, identification of novel predictors for patient outcome and possible therapeutic targets for individualized therapy in ICC is urgently required.

In recent years, the importance of tumor microenvironment in tumor progression has been increasingly recognized. Cancer-associated fibroblasts (CAFs), which are defined as the fibroblastic cells found in a tumor [5], are the most prominent cell type within the tumor microenvironment and have been demonstrated to be a facilitator of tumor growth and progression in a variety of human cancers, including colorectal cancer [6], breast cancer [6], pancreatic cancer [7], and esophageal squamous cell carcinoma (ESCC) [8].

Although CAFs are sometimes referred to as myofibroblasts, they are actually a highly heterogeneous cell population. Morphologically, CAFs are comprised of at least two cell types: myofibroblasts and normal fibroblast-like cells [9]. Myofibroblasts are characterized by large spindle-shaped cells with indented nuclei, while the latter cell type has a thin and small spindle shape of normal fibroblast. Both subtypes of CAFs have been reported to have tumor-promoting activities in mice models [10]. However, few studies have focused on the clinical significance of CAFs based on the histological morphology.

Apart from the spindle-like morphology, several markers have also been suggested to define CAFs, such as α-smooth muscle actin (α-SMA) [11], fibroblast specific protein 1 (FSP-1) [12], and platelet-derived growth factor receptor β (PDGFRβ) [13]. α-SMA is a widely used marker to identify myofibroblasts [14]. FSP-1, also known as S100A4, is another common marker of CAFs and has been indicated to identify a unique population of CAFs distinct from α-SMA−positive myofibroblasts [15]. Besides, PDGFR, including PDGFRα and PDGFRβ, is a kind of tyrosine kinase receptor, which is mainly expressed in fibroblasts. PDGFRβ expression seems to be more common in general [16]. Despite all the markers, molecular definition of CAFs remains a challenge due to the lack of specific and exclusive markers. However, increasing studies have demonstrated that these markers have promising prognostic value. For example, in colorectal cancer, breast cancer, ESCC, and prostate cancer, expressions of α-SMA, FSP-1 or PDGFRβ in CAFs are associated with aggressive feature and poor prognosis [8,17-19].

ICC is a highly malignant neoplasm with excessive stromal desmoplastic reaction. In the desmoplastic stroma of ICC, there are abundant ECM proteins and α-SMA−positive CAFs surrounding the malignant ducts, glands or nests. Given the abundance of CAFs and the tumor-promoting role CAFs played in other cancer types, the impact of CAFs on ICC might be also considerable. However, studies with regard to histological morphology and molecular markers of CAFs in ICC are still limited, and their clinical relevance has not been fully characterized. Thus, this study aimed to evaluate a histological classification of CAFs and the expression of three CAF markers, α-SMA, FSP-1, and PDGFRβ, and their relationship with clinicopathological factors and prognosis in ICC.

2. Materials and methods

2.1. Patients

Formalin-fixed, paraffin-embedded tumor samples were collected from patients who underwent surgical resection for ICC at the Third Affiliated Hospital of Sun Yat-Sen University from 2010 to 2015. Clinicopathological data were obtained from the archives of the Department of Pathology at the Third Affiliated Hospital. All patients were staged according to seventh American Joint Committee on Cancer TNM staging system for ICC [20]. Tumor histological types and differentiation were categorized based on the grading system described by the World Health Organization classification [21]. Follow-up information was obtained by telephone to confirm the status of the patients by the time of December 31, 2015, and the date of death during the follow-up period. This study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University. Written informed consent was collected from all patients before surgery.

2.2. Classification of CAFs by histology

CAF classification was evaluated by two pathologists who had no prior knowledge of clinicopathological data. According to their morphology on hematoxylin and eosin (HE) slides, fibroblasts were classified into mature and immature groups. Thin, wavy, and small spindle-shaped fibroblasts were defined as mature fibroblasts; fat, plump spindle-shaped fibroblasts with one or two nucleoli were regarded as immature fibroblasts (Fig. 1A and B). When the immature fibroblasts accounted for more than 50% of the total fibroblasts within the tumor stoma, the case was considered as immature CAF phenotype; otherwise, it was regarded as mature CAF phenotype [8].

2.3. Immunohistochemical staining

The HE slides were reviewed to choose the representative tumor area for immunohistochemistry (IHC). Then 4-μm-thick sections were cut from the corresponding paraffin-embedded tissue blocks and dried at 65°C for 2 hours for IHC staining. We performed the IHC assay using the two-step EnVision procedure (Dako, Glostrup, Denmark). Sections