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Original contribution

ACOT1 expression is associated with poor prognosis in gastric adenocarcinoma☆,☆☆

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Summary Acyl-CoA thioesterase 1 (ACOT1) is an important isoform of the ACOT family that catalyzes the reaction of fatty acyl–CoAs to CoA-SH and free fatty acids. Recent studies of gastrointestinal tumor metabolism suggest that there is abnormal metabolism of lipids and fatty acids during tumor progression. However, the function and contribution of ACOT1 in gastric cancer development are still poorly understood. In addition, GLI3 is a major transcription factor in the regulation of hedgehog signaling. *GLI3* mutations induce glandular expansion and intestinal metaplasia in gastric cancer, which indicates a role for GLI3 in the preneoplastic process. Thus, we investigated the relationship between ACOT1 expression and GLI3 in gastric adenocarcinoma. A tissue microarray was constructed from 280 cases of gastric adenocarcinoma. The immunohistochemistry method was performed on tissue sections of 4 μm from each tissue microarray block. We found a significant correlation between ACOT1 expression and poor histologic grade, a lower T category, TNM stage, and increased GLI3 expression. In addition, the survival analysis revealed that the ACOT1-positive group had significantly decreased overall survival rates compared with the ACOT1-negative group. Furthermore, GLI3 expression had a significant positive correlation with ACOT1 expression in gastric adenocarcinoma cells. In summary, these findings demonstrate that increased expression of ACOT1 is correlated with pivotal clinicopathological parameters and poor prognosis in gastric adenocarcinoma through increased expression of the potential tumor-promoting protein GLI3.

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1. Introduction

Gastric carcinoma is one of the most common malignancies in the world, but it is especially common in China. Gastric carcinoma is the second leading cause of cancer-related deaths globally [1]. In addition, recent metabolic studies in tumor cells determined that abnormal changes in the pathways of carbohydrate and lipid metabolism resulted in aerobic glycolysis (Warburg effect) and increased the rates of macromolecule synthesis [2,3]. Acyl-CoA thioesterases (ACOTs) are enzymes that catalyze the hydrolysis of fatty acyl–CoA to CoA-SH and free fatty acids [4]. ACOTs are widely distributed in a variety of human tissues. Several ACOT isoforms are localized to distinct cellular organelles, including the cytosol, mitochondria, peroxisomes, and endoplasmic reticulum [5]. The type 1 enzymes of ACOTs are ACOT1-6. ACOT1 is localized to the cytosol, ACOT2 is in localized to the mitochondria, and ACOT3 to ACOT6 are localized to peroxisomes [6]. ACOT1 is primarily involved in the catalytic hydrolysis of arachidonyl-CoA to arachidonic acid and CoA [7]. Arachidonic acid is the major precursor for proinflammatory eicosanoids, and it plays several important cellular roles in cell signaling, the activation of metabolic enzymes, and the regulation of cell proliferation [8,9]. It has recently been recognized that abnormal lipid metabolism plays a role in the metabolism of fatty acids in cancer cells [10,11]. Thus, it is of great significance to investigate ACOT1 as a metabolizing enzyme encoding essential fatty acids in tumor metabolism.

GLI family zinc finger 3 (GLI3) is a member of the Forkhead family of transcription factors that mediate the hedgehog (Hh) signaling pathway [12]. Hh proteins control morphogenesis by promoting GLI-dependent transcriptional activation and inhibiting the transcriptional repressor of GLI3 [13]. Hh regulates differentiation and proliferation of cancer tissue smooth muscle progenitor cells during cancer development [14]. Therefore, there has been considerable interest in the function of the *GLI3* gene in oncogenesis and the mechanisms that modulate GLI3 expression. Madison et al [15] demonstrated that GLI3 expression in carcinoma cells is associated with metastatic adenocarcinomas and suggested that GLI3 expression might be related to tumor proliferation potential and poor survival prognosis.

Both the role of ACOT1 and its significance remain inadequately studied in gastric adenocarcinoma. Recently, Hung et al [16] demonstrated that aberrantly high expression of ACOTs promoted adenocarcinoma proliferation and inhibited differentiation. However, the mechanism of GLI3 overexpression in tumor tissue, its influence on prognosis, and the function of Hh/GLI3 in gastric adenocarcinoma remain elusive [17]. Therefore, we investigated the expression of ACOT1 and its association with GLI3 and other important tumor proliferation and metastasis biomarkers, Ki-67 and erbB2, in gastric adenocarcinoma [18].

This study aimed to investigate the clinical significance of ACOT1 in gastric adenocarcinoma, and it aimed to investigate its relationship with the Hh signaling pathway that controls cell proliferation. A better understanding of GLI3 might produce new therapeutic strategies against tumor progression.

2. Materials and methods

2.1. Patients and tissue samples

Tissue samples were obtained from 280 cases of gastric adenocarcinoma that underwent major surgery at the Affiliated Hospital of Jiangnan University between 2006 and 2011. For each case, 2 researchers reviewed all of the original hematoxylin and eosin–stained sections. The clinicopathological factors analyzed included patient sex, age, tumor size, histologic grade, primary tumor (pT), nodal (pN) metastasis, pathologic stage, vascular invasion, neural invasion, lymphatic invasion, and Lauren classification. The mean follow-up duration was 50.0 months and ranged from 0.8 to 104 months. This study was approved by the ethics review board at the Affiliated Hospital of Jiangnan University.

2.2. Tissue microarray construction

The hematoxylin and eosin–stained sections of formalin-fixed, paraffin-embedded tumor tissue blocks were screened to validate cancerous tissue and adjacent normal tissue of gastric adenocarcinoma. The corresponding spots on the tissue block were then marked for tissue core punch. Tissue microarrays (TMAs) were assembled using manual TMA spotting (Quick-Ray; UNITMA Co, Ltd, Seoul, Korea). Two representative tumor cores with a diameter of 2.0 mm were procured from each gastric adenocarcinoma tissue block. We arranged 2 tissue cores per case to increase the concordance rate between the immunohistochemistry (IHC) results of the TMAs and the integral sections. Each of the TMA cores also contained 2 normal gastric tissue cores from the same block and was arranged according to the corresponding tumor core below. Hematoxylin and eosin staining was done for each block to confirm tumor tissue integrity and cell morphology. Cases with inadequate carcinoma tissue or cases that did not contain adenocarcinoma tissue in the cores were not included.

2.3. IHC staining

IHC was done on 4-μm tissue sections from each TMA block using the Leica paraffin slicer RM2235 (LeicaBiosystems, Nußloch, Germany) according to the manufacturer's protocol with modifications. Briefly, 4-μm sections of formalin-fixed, paraffin-embedded tissue were deparaffinized