ROS kinase fusions are not common in Chinese patients with cholangiocarcinoma

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Abstract: Objective To investigate the expressions of different forms of ROS fusions in Chinese patients with cholangiocarcinoma (CCA). Methods RT-PCR was employed to examine formalin-fixed and paraffin-embedded CCA samples from stage I-IV patients for detection of ROS fusions involving Fused in Glioblastoma (FIG), solute carrier protein (SLC34A2) and major histocompatibility complex class II invariant chain (CD74). Serpin peptidase inhibitor clade A member 1 (SERPINA1) was detected as the reference gene. Results In all the 56 CCA samples, 80.4% (45/56) were positive for SERPINA1 expression as evaluable samples. Of these evaluable samples, none expressed the ROS fusions. Conclusion ROS fusions are not common in Chinese CCA patients.

Key words: ROS fusions; cholangiocarcinoma; reverse transcriptional polymerase chain reaction

INTRODUCTION

Cholangiocarcinoma (CCA) is a relatively rare but highly lethal malignancy arising from the biliary tract epithelium, and an increasing incidence of CCA has been reported worldwide in recent years[1]. The cure for CCA is attainable by curative radical surgical resection or liver transplantation, but the difficulty in early diagnosis often results in inoperable cases and hence a poor outcome of the patients with a 5-year survival of less than 5% and a median survival period of 6 months in advanced cases[2].

Targeted therapy provides a novel strategy to improve the therapeutic efficacy for malignant neoplasms. New molecular targets have been explored for potential CCA therapy. ErbB family has a reported positivity rate ranging from 10% to 81% in CCA patients and its inhibitors can suppress cellular growth and induce apoptosis[3]; c-Met and its cascade (such as MEK1/2) were also investigated as potential therapeutic targets for CCA[4].

The receptor tyrosine kinase ROS is a member of the src family[5] and have been detected in a variety of human cancers[6-9]. According to El-Deeb et al.[10], the mutant forms of ROS are derived mainly from its fusions with such proteins as Fused in Glioblastoma (FIG), solute carrier protein (SLC34A2), and major histocompatibility complex class II invariant chain (CD74). Both FIG-ROS fusion and SLC34A2-ROS fusion have two different forms. FIG-ROS (S) is the result of fusion of exon 3 of FIG to exon 36 of ROS, FIG-ROS(L) the result of fusion of exon 7 of FIG to exon 35 of ROS, and they were reported to be oncogenic in glioblastoma[11-12] and CCA[13]. Similarly, SLC34A2-ROS (S) is derived from fusion of exon 4 of SLC34A2 to exon 34 of ROS, and SLC34A2-ROS (L) from fusion of exon 4 of SLC34A2 to exon 32 of ROS, both of which were found in lung cancer cells and tissues[6]. Another ROS mutant CD74-ROS is the result of fusion of exon 6 of CD74 to exon 34 of ROS, which has been shown to accelerate lung cancer growth[6,14].

ROS kinase inhibitors are capable of inhibiting ROS fusion proteins due to the conservation of the intracellular tyrosine kinase domain[10]. Data have shown that the multi-targeted tyrosine kinase inhibitor, crizotinib, can inhibit the proliferation of ROS-rearranged lung cancer cells and lead to tumor reduction in patients with advanced NSCLC who have ROS rearrangement, suggesting the potential of crizotinib in treatment of cancers positive for ROS fusion proteins[15-18]. Other highly selective ROS kinase inhibitors are also explored. El-Deeb et al.[19] reported a highly selective inhibitor pyrazole compound 1, which could specifically inhibit the enzymatic activity of ROS kinase with an inhibition rate reaching 94% at a single dose of 10 mmol/L.

ROS fusions have been shown to promote tumorigenesis and progression, and their inhibitors may be promising for molecular-targeted therapy in CCA, but their role in CCA remains unknown. The only available study addressing ROS fusions in CCA was conducted by Gu et al.[11], who found the presence of ROS fusions in 8.7% (2 out of 23) of CCA patients. In this study, we detected the mRNA expression of ROS fusions including FIG-ROS, SLC34A2-ROS, and CD74-ROS in 56 CCA samples to investigate the prevalence of ROS fusions in Chinese patients with CCA.
MATERIALS AND METHODS

Patients and tissue samples

Formalin-fixed and paraffin-embedded (FFPE) CCA samples were collected from CCA patients undergoing surgery in our hospital, Second Affiliated Hospital of Guangzhou Medical University and Guangzhou Medical University Cancer Institute and Hospital between 2001 and 2011. This study was approved by the Institutional Review Board of these Hospitals.

RNA Extraction

All the tissues were identified pathologically with HE staining, and the tumor areas were marked on the tissue slides. After dissecting out the marked tumor area, the total RNA of the samples was extracted using RNeasy FFPE Kit (Qiagen, Dusselddorf, Germany) according to the manufacturer’s instructions.

RT-PCR

cDNA was generated by reverse transcription using a high-capacity RNA-to-cDNA Master Mix (ABI, Carlsbad, California, USA). The primers used to amplify ROS fusions and serpin peptidase inhibitor clade A member 1 (SERPINA1) were listed in Tab.1. AmpliTaq Gold 360 Master Mix (ABI, Carlsbad, California, USA) was used for PCR reaction following the manufacturer’s protocols. PCR amplification was performed in 40 thermal cycles (94 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s). To sequence the positive PCR products, we added the sequencing primer tags (Forward: 5'-actgtaaaacgacggccagt + gene specific primer-3'; Reverse: 5'-accaggaaacagctatgacc + gene specific primer-3') to the gene-specific primers, which lengthened the products by 40 bp. The product sizes of FIG-ROS(S), FIG-ROS(L), SLC34A2-ROS(S), SLC34A2-ROS(L), and CD74-ROS were 91 bp, 94 bp, 142 bp, 144 bp, and 134 bp, respectively.

RESULTS

Fifty-six samples were obtained from 56 CCA patients (including 22 male and 34 female patients). The general demographic and clinical data of the patients are listed in Tab.2. The tumor stage was defined according to the 6th edition of the AJCC Cancer Staging Manual (2010). Of these patients, 26 in stages I-III received radical resection, and 30 stage IV patients underwent palliative surgery.

Expression of SERPINA1

To test the quality of our FFPE samples, SERPINA1 was detected as the reference gene in all the 56 FFPE samples. We found that 80.4% (45/56) of the samples expressed SERPINA1 (with a size of 77 bp, Fig.1) and were therefore eligible for further testing.

Expression of FIG-ROS(S), FIG-ROS(L)

Different from the previous study, we did not find the expression of FIG-ROS(S) (Fig.2A) or FIG-ROS(L) (Fig.2B) in the 45 evaluable CCA samples. The expected product sizes of FIG-ROS(S) and FIG-ROS(L) were 131 bp and 134 bp, respectively.

Expression of SLC34A2-ROS(S), SLC34A2-ROS(L)

RT-PCR was performed on the 45 assessable samples to survey the frequency of expression of SLC34A2-ROS fusions in Chinese CCA patients. Neither SLC34A2-ROS(S) (182 bp, Fig.3A) fusion nor
SLC34A2-ROS(L) (184 bp, Fig.3B) fusion was detected in these samples.

Expression of CD74-ROS

The results of RT-PCR showed that none of the 45 assessable samples expressed CD74-ROS fusion (with an expected product size of 174 bp, Fig.4A).

Expression of ROS fusions in 12 representative samples

To observe the relative position of the ROS fusions and reference gene, we detected the expressions of the 5 ROS fusion genes and the reference gene in 12 representative samples (Fig.4B). ROS fusions involving FIG, SLC34A2, or CD74 were not detected.

DISCUSSION

ROS fusions were reported to express primarily in glioblastoma and NSCLC to promote their tumorigenesis and progression \[6, 10, 20-21\]. Among the CCA samples we obtained, none of them showed positive mRNA expressions for FIG-ROS, SLC34A2-ROS or CD74-ROS fusions. Gu et al detected FIG-ROS fusions in only 2 out of 23 Chinese CCA patients \[13\]. To detect the expression of ROS fusions, we used RT-PCR to amplify the mRNAs from the 56 FFPE CCA samples. Although mRNA is easily degraded in FFPE samples, the fragments between 100 and 200 bases in length can still be conserved \[22\]. Studies \[23-24\] have shown that the PCR product less than 200 bp can be visible on the gel, and we therefore designed the size of the PCR products to be less than 200 bp. SERPINA1 was used as the reference gene to ensure the quality of the FFPE samples, and finally 45 samples were found to be evaluable in this study.

In this study, no FIG-ROS fusions were detected in the 45 evaluable samples. This result is different from that reported by Gu et al, who detected FIG-ROS fusions in 2 out of 22 CCA samples with one expressing FIG-ROS(L) and the other expressing FIG-ROS(S). In this context, we can not exclude the possibility of the presence of FIG-ROS fusion in Chinese CCA patients. We also failed to detect the expressions of SLC34A2-ROS and CD74-ROS fusions in the CCA samples, and this result is consistent with the previous reports that SLC34A2-ROS and CD74-ROS fusions were seldom or never expressed in tumors \[13\]. In a study conducted among 202 Asian never-smoking patients with lung adenocarcinomas, Li et al \[25\] detected the expressions of SLC34A2-ROS and CD74-ROS fusions in 0 and 2 (1%) of the patients, respectively \[25\]. Rimkunas et al \[26\] also found that only 1.6% (9 out of 556) of the NSCLC patients had positive expression of ROS1, which
was subsequently confirmed as FIG-ROS, SLC34A2-ROS, and CD74-ROS by RT-PCR. They concluded that NSCLC tumors with ROS rearrangements were uncommon in Chinese population. In the study by Rikova et al [6] who examined the expressions SLC34A2-ROS (including the long form and short form) and CD74-ROS fusions in 41 lung cancer cell lines and 150 human NSCLC samples, only HCC78 cell line and one NSCLC sample were found to have a positive expression. A similar result was also obtained in a study conducted among lung cancer patients in Japan, which reported a positivity rate of only 0.7% (11/1476) in the 1476 patients [21]. All these data, combined with our results, suggest very low, if any, expressions of SLC34A2-ROS, CD74-ROS, and FIG-ROS fusions in Chinese CCA patients.

In summary, although inhibition of ROS fusions appears to be a promising therapeutic strategy for tumors, the very low (if not zero) expression rates of ROS fusions (FIG-ROS, SLC34A2-ROS, and CD74-ROS) in our CCA samples suggest its limited potential clinical application in Chinese CCA patients.

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