The specific serotonin reuptake inhibitors sertraline and fluoxetine promote tumor growth in a mouse xenograft model of cholangiocarcinoma

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Abstract
Cholangiocarcinoma is an aggressive cancer of the bile ducts with limited treatment options and a high mortality rate. Patients with cancer have over a twofold higher rate of depression than the general population and are prescribed selective serotonin reuptake inhibitors (SSRIs) to manage their depression. However, serotonin has been demonstrated to promote the proliferation of cancer cells. In this mouse xenograft study, we provide evidence that the SSRIs sertraline and fluoxetine lead to increased serotonin bioavailability that results in a proliferation of cholangiocarcinoma cells. Cholangiocarcinoma cell proliferation was not observed in vitro, indicating that the effects of SSRIs are due to increased serotonin levels in noncancerous cells of the body. These novel findings on the effect of SSRIs in promoting the growth of cholangiocarcinoma support the notion that these antidepressants should be used cautiously in this patient population.

Introduction
Cancer of the bile ducts, or cholangiocarcinoma, is an extremely aggressive tumor that has very poor prognosis and limited treatment options [1] [2]. Cholangiocarcinomas accounts for around 15% of all liver cancers and causes 2% of all cancer deaths worldwide [3]. Despite aggressive treatment, survival rates are low, generally only 6 months from diagnosis, as 90% of patients are not eligible for surgery-, and the cancer is relatively resistant to chemotherapy. The high mortality rate from cholangiocarcinoma is due in part to the late diagnosis, as the clinical manifestations of this cancer (such as abdominal pain, pruritus, weight loss, etc.) occur after the cancer is quite advanced [4].

Serotonin, or 5-hydroxytryptamine (5-HT), is synthesized via the decarboxylation and hydroxylation of tryptophan by the enzymes tryptophan hydroxylase and decarboxylase [5]. Serotonin can then generate a wide variety of intracellular effects as there are 16 receptor subtypes, with all but one being G protein–coupled receptors that lead to the activation of secondary messenger systems [6]. The uptake of serotonin from the extracellular space into the cell via serotonin transporters terminates serotonin receptor-mediated signaling [7]. Predominantly, it is the enzyme monoamine oxidase A that degrades serotonin once it is inside the cell [8]. Serotonin has been classified as a growth factor for several different nontumorigenic cell types as well as in a variety of cancer cells, including small cell lung carcinoma, choriocarcinoma, bladder cancer, prostate cancer, hepatocellular carcinoma, and breast cancer [9] [10] [11] [12] [13] [14] [15]. Similarly, cholangiocarcinoma produces increased amounts of serotonin, [16] which exerts growth-promoting effects in vitro and in vivo. This increased biogenic amine production is due to the coordinated increase in synthesis and the epigenetic silencing of monoamine oxidase A [16] [17] [18].

Cancer patients have a variety of stressors (physiological, monetary, and psychological) that result in clinical depression at rates much higher than the general population [19]. The incidence of depression in the general population has been reported to be between 5% and 6%, whereas in cancer patients this jumps to 12.9%, with another 16.5% displaying subclinical depressive symptoms [20] [21]. The use of SSRIs for the management of depressive symptoms has become more common in recent years with antidepressant use increasing nearly 400% in the time period of 2005–2008 when compared to 1988–1994 [22]. As SSRI antidepressants work through inhibiting serotonin transporter activity,
The specific serotonin reuptake inhibitors sertraline and fluoxetine promote tumor growth in a mouse xenograft model of cholangiocarcinoma. Their use leads to increased serotonin bioavailability. The effects of SSRI use on cholangiocarcinoma growth have not yet been determined.

Objective
The objective of this study was to assess the effects of the SSRIs sertraline and fluoxetine on cholangiocarcinoma growth using a xenograft tumor model in mice.

Figure Legend
Figure 1.
(A) Nude mice were injected with the Mz-ChA-1 cholangiocarcinoma cell line, and a xenograft tumor was established. These mice were then treated with sertraline (20 mg/kg/day) or fluoxetine (10 mg/kg/day) 3 times per week, and the tumor volume was measured using digital calipers. Data are expressed as avg ± SEM of the tumor volume.
(B) Representative images of Mz-ChA-1 tumors excised at 24 days following xenograft tumor establishment from vehicle-, sertraline-, and fluoxetine-treated mice.
(C) Xenograft tumor sections were stained with the cholangiocyte marker cytokeratin...
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Results & Discussion
The tumors from mice treated with sertraline or fluoxetine were significantly larger than those treated with vehicle and grew quicker over time (Fig. 1A and B). Immunohistochemistry for the cholangiocyte marker CK-19 was performed to determine whether the resulting tumors had similar cellular makeup and tumor architecture. Across all treatments, the majority of cells in the tumors were CK-19 positive tumor cells with similar morphology and a similar degree of non-tumor cells (Fig. 1C). However, in tumors from mice treated with sertraline or fluoxetine, there were increased numbers of PCNA-positive cells (Fig. 1C) and an increased expression of PCNA mRNA (Fig. 1D), indicating a greater degree of xenograft cell proliferation in response to SSRI treatment. Interestingly, when cholangiocarcinoma cells were treated with fluoxetine and sertraline in vitro, there was no effect on cell proliferation (Fig. 1E) or cell cycle progression (Fig. 1F), suggesting that the proliferative effects observed in vivo are more likely due to indirect action on other cells that make up the tumor microenvironment or even other organs in the body rather than direct action of these SSRIs on serotonin reuptake on the tumor cells themselves. In support of this, treatment of mice with the selected SSRIs significantly increased the serotonin levels in the serum (Fig. 1G).

Conclusions
The data presented here suggest that treatment of cholangiocarcinoma xenograft-bearing mice with sertraline or fluoxetine increased tumor growth. These data may indicate that the use of SSRI antidepressants may be contraindicated for patients with cholangiocarcinoma.

Limitations
The limitations of these observations may include the following:

1. Not all SSRIs are equal: Our study only assessed two of the main SSRIs; however, there are many alternative SSRIs and antidepressants that work through other mechanisms, such as monoamine oxidase inhibitors or tricyclic antidepressants, which may have differing effects on cholangiocarcinoma growth. Further characterization of these antidepressants should be assessed.

2. Not all species are equal: In this study, mice were given sertraline and fluoxetine 3 times per week at concentrations of 20 mg/kg and 10 mg/kg, respectively. These were the concentrations used in previous studies to assess their effects on depression-like behaviors in mice [23] [24]. These dosages are in excess of the recommended daily dose in humans (Fluoxetine; 20–80 mg per day; assuming an 80 kg person, corresponds to approximately 0.25–1 mg/kg/day. Sertraline; 50–200 mg/day, corresponds to approximately 0.625–2.5 mg/kg/day). However, it is difficult to apply similar doses to humans...
and mice, as the relative pharmacokinetics of these antidepressants in each species is different.

3. The molecular or cellular target of the SSRIs is not identified: In this study, the effects of SSRIs on cholangiocarcinoma cell proliferation in vitro was negligible, leading to the suggestion that the effects of SSRIs on tumor growth in vivo are likely due to effects either on other cells that may contribute to the tumor microenvironment or perhaps other organs that serve to bring about an increase in systemic serotonin levels. This study did not identify the precise cellular target of these SSRIs, and this topic is one that merits ongoing investigation.

Additional Information

Methods and Supplementary Material
Please see https://sciencematters.io/articles/201706000003.

Funding Statement
This study was supported by an American Cancer Society Research Scholar award (RSC118760), an NIH R01 award (DK082435), and a VA Merit award (BX002638) from the United States Department of Veterans Affairs Biomedical Laboratory Research and Development Service to Dr. DeMorrow. This study was also funded by a VA Career Development award (BX003486) from the United States Department of Veterans Affairs Biomedical Laboratory Research and Development Service to Dr. McMillin.

Acknowledgements
This work was completed with support from the Veterans Health Administration and with resources and the use of facilities at the Central Texas Veterans Health Care System, Temple, Texas. The contents do not represent the views of the United States Department of Veterans Affairs or the United States Government.

Ethics Statement
Animal experiments were performed with approval from the Baylor Scott & White Institutional Animal Care and Use Committee (protocol no: 2012-051).

Citations


