Pancreatic cancer (PaCa) is known to be one of the most lethal human cancers with <5% 5-year survival rate because of its strong invasive capacity with frequent metastasis and recurrence. Novel molecular targeted therapies need to be developed to overcome such an aggressive tumor cells. Serotonin (5-HT), a mitogenic neurotransmitter, was previously known to act as a growth factor for several types of cells including tumor cells. Recently, 5-HT has emerged as an important regulator of cell proliferation and tumor growth in a variety of cancer types and often up-regulated in tumor progression. However, there is lack of evidence about the impact of 5-HT receptors on promoting pancreatic cancer. Given the important role of 5-HT-1 receptors, especially 5-HT1B and 5-HT1D subtypes, in different types of malignancies, the aim of this study was to investigate the role of 5-HT1B and 5-HT1D receptors in PaCa growth and progression and analyze their potential as cytotoxic targets. We found that knockdown of 5-HT1B and 5-HT1D receptors expression using specific small interfering RNA (siRNA) induced significant inhibition of proliferation and clonogenicity of PaCa cells. In conclusion, our data suggests that 5-HT1B– and 5-HT1D receptors mediated signaling play an important role in the regulation of the proliferative and invasive phenotype of PaCa. It also highlights the therapeutic potential of targeting of 5-HT1B/1D receptors in the treatment of PaCa, and opens a new avenue for biomarkers identification, and valuable new therapeutic targets for managing pancreatic cancer.