Pancreatic ductal adenocarcinoma is one of the most lethal cancers with extensive local tumor invasion, metastasis, early systemic dissemination, and poorest prognosis. Thus, finding novel targets for pancreatic cancer (PaCa) therapy are urgently needed. Eukaryotic elongation factor-2 kinase (eEF-2K) is an atypical kinase that we found to be highly up-regulated in PaCa cells. However, its role in PaCa progression/invasion remains unknown. Here, we investigated the role of eEF-2K in cells invasion, and we found that down-regulation of eEF-2K, by siRNA or rottlerin (4-6 µM), displays impairment of PaCa cells invasion, with significant decreases in the expression of tissue transglutaminase (TG2), the multifunctional enzyme implicated in regulation of cell attachment, motility and survival. These events were associated with reductions in β1 integrin and uPAR expressions as well as decrease in Src activity. In addition, inhibition of eEF-2K/TG2 axis suppresses the epithelial-mesenchymal transition (EMT), as evidenced by the modulation of the zinc finger transcription factors, ZEB1 and Snail, and the tight junction protein, claudin-1. Importantly, while the silencing of eEF-2K recapitulates the rottlerin-induced inhibition of invasion and correlated events, eEF-2K over-expression, by lentivirus-based expression system, suppresses such rottlerin effects and potentiates the invasion capability of PaCa cells. Collectively, our results suggest, for the first time, that eEF-2K is involved in regulation of the invasive phenotype of PaCa cells through promoting a new signaling pathway, which is mediated by TG2/β1 integrin/Src/uPAR, and the induction of EMT biomarkers which enhances cancer cell motility and metastatic potential. Thus eEF-2K could represent a novel potential therapeutic target in pancreatic cancer.