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Aspirin and gemcitabine synergistically inhibit pancreatic cancer stem cell features through inhibition of NF-κB signaling

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Pancreatic cancer is one of the worst malignancies and the fourth leading lethal cause of cancer deaths in the USA. Gemcitabine (GEM) is used for treatment of advanced pancreatic cancer, although only less than 25 % of patients benefit from it. Cancer stem cells (CSCs) are considered to be responsible for the high therapy resistance and tumor relapse. Of note, the numbers of CSCs in the tumor even enriched after treatment with GEM. Constitutive activation of NF-kappaB (NF-κB) signaling has been found to play an important role in regulating the CSCs properties and maintaining therapy resistance. It is well known that inflammation plays crucial roles in tumor progression. Increasing clinical and epidemic evidences indicate that nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin (ASA) reduce incidence, metastasis, and cancer-specific mortality. These data suggest that ASA may be a potential agent for targeting of CSCs. In the present study, I evaluated the effect of ASA on pancreatic CSCs characteristics, estimated whether ASA sensitizes pancreatic CSCs to GEM and examined the underlying mechanisms.

I found that ASA strongly reduced viability even at low concentration (2 mM) in CSC^{high} cells (AsPC-1 and PANC-1), CSC^{median} cells (established GEM-resistant BxPc-3/GEM), and CSC^{low} cells (BxPc-3), with stronger effects in CSC^{high} cells. In contrast, GEM (50 nM) at a therapeutic concentration had only marginal effects in CSC^{high} cells. However, co-treatment with ASA (5 mM) significantly reduced viability of both CSC^{high} and CSC^{median} cells. Similarly, ASA pronouncedly enhanced pro-apoptosis effect of GEM particularly in GEM-resistant CSC^{high} cells. Next, I estimated the effect of ASA on CSCs characteristics and the results showed that ASA (5 mM) reduced the potential of pancreatic CSCs to form colonies along with spheres and this potential was almost completely abolished when combined with GEM. In addition, ASA also significantly inhibited expression of CSCs marker Nang, Oct-4, and SOX2, c-Met, Notch-1and its ligands Jagged-1, and CD133. Importantly, the strongest effects were observed upon combination of ASA and GEM, while GEM alone was less effective and even induced the amount of CSC marker-positive cells. Similarly, ASA alone or combined with GEM significantly reduced ALDH1 expression and activity. Furthermore, ASA effectively inhibited epithelial-mesenchymal-transition (EMT) by up-regulation of E-Cadherin and downregulation of Snail and Twist2. These results were supported by in vivo tumor xenograft studies, which showed that ASA together with GEM strongly inhibited tumor take, growth and invasion.

To investigate whether ASA sensitizes pancreatic CSCs to GEM by the inhibition of NF-κB survival signaling, EMSA was performed to determine the binding activity of NF-κB subunits. The results showed that GEM significantly reduced the binding activity of NF-κB in CSC^{low} cells, whereas it strongly induced it in CSC^{high} cells. However, ASA alone not only remarkably inhibited NF-κB activity, but also totally abolished GEM-induced NF-κB activity in CSC^{high} cells. Correspondingly, western blot results demonstrated that ASA, but not GEM, led to a strong down-regulated expression of the NF-κB subunits p65, p52, and c-Rel and combined treatment nearly totally abrogated the expression of these proteins. Because NF-κB activity is associated with inflammation and inhibition of inflammation is a well-known function of ASA, western blot and immunohistochemistry were performed to investigate

whether ASA inhibits the expression of the inflammatory factor tumor necrosis factor-α (TNF-α). Indeed, ASA strongly diminished TNF-α expression while GEM distinctly induced TNF-α expression, but combined treatment almost completely inhibited TNF-α expression.

In order to examine whether a combination of ASA with GEM is toxic to normal cells, human pancreatic duct cells (CRL-4023) and mesenchymal stem cells (MSCs) were treated. GEM alone had pronounced toxicity in both MSC and CRL-4023 cells, in contrast to ASA, which had no obvious toxic effects. More importantly, the toxicity upon combination of ASA and GEM was significantly decreased compared with GEM treatment alone.

In conclusion, this study provides evidence that ASA not only has significant anti-CSCs effects, but strongly reverses chemotherapy resistance of established pancreatic CSCs to GEM-mediated cytotoxicity through abolishing of GEM-induced NF-κB activity. Most importantly, compared with other chemotherapeutics, ASA has no obvious toxic effects on normal cells and significantly reduces GEM-mediated toxic side effects. Besides that, ASA also has other advantages such as easy administration and cardiovascular protection. Thus, ASA may become a promising adjuvant agent to improve the efficacy and safety of GEM in patients with pancreatic cancer.