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Tissue Transglutaminase Downregulation Potentiates Gemcitabine Efficacy and Blocks Pancreatic Cancer Growth in vivo

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive neoplastic diseases and is virtually incurable. The molecular mechanisms that contribute to the intrinsic resistance of PDAC to multiple anticancer therapies are not well understood. We observed that several drug-resistant (including gemcitabine) and metastatic tumors and tumor cell lines express elevated levels of tissue transglutaminase (TG2). TG2 is a ubiquitous enzyme that, in addition to catalyzing Ca²⁺-dependent post-translational modification of proteins, can also serve as a Ca²⁺-independent signaling molecule, leading to the activation phospholipase C/MAP kinase pathways. TG2 also promotes RhoA, focal adhesion kinase, and β -integrin-mediated cell adhesion and cell migration. We showed that elevated expression of TG2 in PDAC cells was associated with increased gemcitabine resistance and invasive potential. Conversely, down-regulation of TG2 by siRNA attenuated gemcitabine resistance and invasive functions in vitro.

Aim: Determine whether liposomal TG2-specific siRNA can block tumor growth and metastasis either alone or in combination with gemcitabine in an orthotopic model of PDAC.

Methods and Results: Using Panc-28 cells (TG2^{hi}) stably transfected with luciferase as our model system, we examined the effects of TG2-specific siRNA (liposomal formulation) in our orthotopic PDAC model in nude mice. The mice were randomized into four groups (n=6/group) after 3 weeks based on luciferase bioluminescence using Xenogen IVIS 200. The four groups consisting of scrambled siRNA, gemcitabine (25 mg/kg/twice weekly; intraperitoneal [IP]), TG2-siRNA (150 µg/kg/thrice weekly; intravenous [IV]), and gemcitabine + TG2-siRNA, were treated for 4 weeks and tumor volumes were measured weekly using bioluminescence imaging. Animals were sacrificed 7 weeks after tumor implantation and tumor volumes were measured. Gemcitabine + TG2-siRNA significantly inhibited growth ($P=.007$ vs. scrambled and $P=.042$ vs. gemcitabine) and also blocked metastasis (by 80%; $P<.001$). Interestingly, TG2-siRNA alone blocked metastasis similar to the combination group. TG2-siRNA either alone or in combination significantly decreased Ki-67⁺ expression ($P<.001$ vs. scrambled and $P<.05$ vs. gemcitabine) and the combination group significantly reduced CD31⁺ microvessel density ($P<.001$ vs. scrambled). Mechanistically, TG2-siRNA either alone or in combination reduced AKT phosphorylation (S⁴⁷³), and blocked NF-κB activity and expression of its downstream gene product, vascular endothelial growth factor (VEGF).

Conclusion: Our results show *for the first time* that TG2-siRNA (liposomal formulation) given intravenously enhanced antitumor effects of gemcitabine by significantly reducing orthotopic PDAC tumor growth and blocking local invasion/metastasis in nude mice. Thus, targeting TG2 in combination with chemotherapeutic agents is a novel therapeutic strategy that improves drug resistance and prevents metastasis of PDAC.