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ABSTRACTS

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Liposome-Formulated siRNA Against Msi1 and Docetaxel Treatment in an Animal Model of Advanced Gastric Adenocarcinoma Eradicates Cancer Stem Cells and Metastasis

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Introduction: In advanced gastric adenocarcinoma, potent chemoresistant cancer stem cells (CSCs) differentiate into progenitor cells that form all of the cell types in the patient's tumor. Cancer stem cells have genetic mutations that render them resistant to chemotherapy- or radiation-induced injury due to rapid repair of DNA damage. The goal of this study was to use antisense molecular targeting to eradicate cancer stem cells, as well as docetaxel, thus eliminating tumor cells and potential recurrence and metastasis in a gastric cancer model.

Materials and Methods: Tumor cells that overexpressed bcl-2 and CSCs were obtained from chemoresistant patients with metastatic advanced gastric adenocarcinoma. The CSCs overexpressed Msi1, which activates Notch and Wnt pathways. The tumor cells were orthotopically transplanted into genetically engineered immunodeficient mice, which developed a tumor with the same cell types as in the human tumor. The animals were treated with pegylated liposomes composed of phospholipids with high transition temperature (Tc). We entrapped docetaxel in the acyl chains, and encapsulated a 21-base pair of small interfering RNA (siRNA) strand targeted to Msi1 in the liposomes. This colloidal formulation was termed LP/AS-Msi1/TXT (under patent).

Results: Post-treatment, the endocytosed siRNA unwound and was incorporated into RNA-induced silencing complex (RISC), which is a stable protein-RNA complex. siRNA was then directed to the targeted Msi1 messenger RNA (mRNA), which is involved in the cancer stem cell pathway. The Msi1

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mRNA undergoes cleavage and degradation, interrupting the protein synthesis of the targeted Msi1 gene. This causes downregulation of Wnt-suppressing cell proliferation, migration of cancer stem cells by inhibiting angiogenesis after VEGF downregulation, and induction of apoptosis after downregulation of antiapoptotic caspase inhibitor survivin. Furthermore, siRNA against Msi1 inhibits degrading proteases of extracellular matrix, such as MMP26 and matrilysin, and cell adhesion molecules, such as neuronal cell adhesion molecule (NRCAM) and CD44, inhibiting invasion and metastasis. Also, blockage of the Wnt signaling cascade led to inhibition of cancer progenitor cells by downregulation of NRSF/REST and ENC1 with BTB-like domain genes. It also blocked tumorigenesis by downregulating claudin1, which leads to inhibition of the Ctnn-Beta-TCF/LEF signaling pathway. Downregulation of Msi1 inhibited the Notch signaling pathway, blocking nuclear transcription factors, downregulating genes, and inhibiting proteins involved in the self-renewal and regeneration of cancer stem cells (which might be considered the roots of the gastric adenocarcinoma tree), leading to their eradication by inhibiting mitotic divisions. Docetaxel treatment, via cell signaling mechanisms, eradicated tumor cells (the leaves of the gastric adenocarcinoma tree) by phosphorylating antiapoptotic oncogene bcl-2, leading to induction of apoptosis, or type I programmed cell death (PCD). Downregulation of bcl-2 led to upregulation of tumor suppressor gene Beclin-1, inducing autophagy, or type II PCD. Polymerization of microtubules led to cell cycle blockage, inhibiting mitosis. BrdU and MTT assays exhibited inhibition of DNA synthesis and metabolic activity, respectively. Polymerization of microtubules led to cell cycle blockage, inhibiting mitosis. Transmission electron microscopy demonstrated a phagocytic bystander killing effect mediated by APCs, and adjacent tumor cells. Finally, we observed morphologic and metabolic evidence of inhibition of tumor recurrence and metastasis on computed tomography and positron emission tomography scans, respectively.

Conclusion: The novel therapy, LP/AS-Msi1/TXT, is designed to target cancer stem cells by inhibiting vital pathways, thus eradicating the “roots” of advanced gastric adenocarcinoma recurrence and metastasis, while the co-administered, conventional chemotherapeutic agent docetaxel eradicates the tumor cells (or the “leaves” of advanced gastric adenocarcinoma). LP/AS-Msi1/TXT represents a potential tailored approach to target cancer stem cells with less toxicity than observed with conventional chemotherapy.