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Inhibition of Epithelial Mesenchymal Transition (EMT) With Immunochemogene Treatment in Metastatic Colorectal Cancer

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Background: Epithelial to mesenchymal transition (EMT) causes resistance to epidermal growth factor receptor (EGFR) inhibitors. We used immunochemogene treatment composed of a stealth nanoparticle formulation, consisting of clamp PNA against mRNA of FOXC2, anti-CD44 chimeric MAb, and vinorelbine, in an attempt to eradicate metastatic colorectal cancer (mCRC) cells and inhibit metastasis by blocking EMT.

Methods: Tumor cells from patients with stage IV chemoresistant CRC characterized by upregulation of FOXC2, CD44, and bcl-2 were obtained surgically. We synthesized antisense clamp peptide nucleic acid (PNA) oligomers (DNA analogs), in which the 6 mer homopyrimidine triplex [(PNA)2/RNA)] hybridized to the 5-end (Leader), and the 10 mer purine/pyrimidine duplex (PNA/RNA) hybridized to the 3-end (Trailer) of the AUG start codon region on the mRNA of FOXC2. The uncharged and hydrophilic antisense clamp PNA anti-FOXC2 was incorporated in the polar phase, and the vinorelbine molecules were entrapped in the acyl-chains of the lipid phase. This was surrounded by the stealth/biocompatibility polymer layer and biological recognition layer with linked chimeric MAbs against CD44 of the nanoparticle formulation. This was used to treat xenograft animal models developed from CRC cells obtained from the stage IV patients. Tumor cells were analyzed with microarray, single-nucleotide polymorphism (SNP) assay, polymerase chain reaction (PCR), western blot (WB), Southern blot (SB), immunoblotting (LC-MS/MS), immunofluorescence staining, immunohistochemistry (IHC), fluorescent activated cell sorter (FACS), confocal microscopy, transmission electron microscopy (TEM), bromodeoxyuridine (BrdU), MTT, and flow cytometry.

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Results: Post-treatment, we observed downregulation of CD44 and Fra-2, and induction of antibody-dependent cellular cytotoxicity (ADCC). The clamp PNA inhibited translation of FOXC2, resulting in activation of Jak2/Stat5a genes, which led to suppression of EMT of cancer cells. This blocked CRC metastatic invasion by reversing the mesenchymal phenotype; reconstituted homotypic adhesion; and promoted differentiation in CRC cells. Undifferentiated epithelial cells undergoing EMT exhibited overexpression of FOXC2, and this expression was lost when these cells returned to their initial differentiated epithelial state, blocking invasion and metastasis. Inhibition of EMT downregulated EGFR and inactivated NF-kB, inhibiting its downstream signaling pathway. Epithelial cell junction proteins claudin 4, claudin 7, and E-cadherin were overexpressed, upregulating beta-catenin; while mesenchymal markers vimentin and fibronectin were downregulated. Downregulation of Twist, Snail, and transcription 3 and 5 blocked the migratory potential of tumor cells, inhibiting metastasis. Calcium-independent cell-cell adhesion molecules EpCAM and TROP2 were upregulated. Vinorelbine blocked tumor cells at G2/M cell cycle, and phosphorylated bcl-2. This circumvented resistance to anoikis, inducing apoptosis in tumor cells due to lack of adhesion, inhibiting invasion and metastasis. In addition to the induction of caspase-dependent apoptosis or programmed cell death (PCD) type I in tumor cells, bcl-2 downregulation caused release of beclin-1 and upregulation of bcl-2–interacting mediator of cell death (BIM), inducing type II PCD or autophagy. TEM exhibited bystander killing effect of tumor cells by adjacent cells, and activated phagocytic cells such as macrophages. DNA synthesis and metabolic activity of tumor cells were inhibited according to BrdU and MTT tests, respectively.

Conclusion: This immunochemogene treatment induced epithelial differentiation by reversing the mesenchymal phenotype, promoted homotypic adhesion, inhibited the multigene signature indicative of EMT, blocking metastatic cell motility/invasiveness, and eradicated mCRC cells resistant to EGFR inhibitors by induction of PCD type-I and type-II, apoptosis and autophagy, leading to a bystander killing effect.