

Metastasis-initiating cells (MICs) recruit and reprogram bystander indolent cells to participate in prostate cancer progression and metastasis

Gina C.Y. Chu, Ruoxiang Wang, Haiyen E. Zhou, Edwin Posadas, and Leland W. K. Chung
Uro-Oncology Research Program, Departments of Medicine and Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA. 90048

Background: Metastasis-initiating cells (MICs) are leader cells that promote metastatic seeding of prostate cancer (PC) cells to bone and soft tissues. MICs found in clinical PC specimens predict the survival of PC patients. MICs were also identified in freshly harvested and *ex vivo* expanded circulating tumor cells (CTCs) from metastatic castration-resistant PC (CRPC) patients by multiplexing quantum dot labeling and RNA *in situ* hybridization analyses. We observed that human PC cells or CTCs with MIC phenotype recruit and reprogram naïve bystander indolent cells to co-express epithelial-to-mesenchymal transition (EMT), stem, and neuroendocrine (NE) phenotypes, together promoting PC progression and metastasis. We discovered among the key transcription factors (TFs) that c-Myc and FOXM1 play dominant roles mediating the communication between MICs and bystander cells. Selective inhibitors of these key TFs blockaded this intercellular communication and deprogrammed the MIC-induced aggressive phenotypes of bystander PC cells.

Methods: Aggressive PC cell lines or CTCs with MIC phenotype were co-cultured with indolent RWPE-1 or primary-tumor-derived DC-1 cells in 3-D suspension culture for several days. Programmed RWPE-1 or DC-1 cells were separated from MICs by FACS and analyzed for their acquired MIC phenotypes and behaviors *in vitro* and *in vivo*. Common upstream TFs were identified by RNA sequencing and computational analysis and methylation status of the programmed cells were analyzed by EPIC methylation array.

Results: We demonstrated that programmed DC-1 and RWPE-1 cells expressed elevated MIC signature markers, displayed increased growth, survival and invasive characteristics, and formed tumors in mice. MIC-induced reprogramming of indolent PC cells is mediated by activation of c-Myc and FOXM1 through NRP1 signaling, leading to epigenetic demethylation of some MIC-associated gene promoters in the programmed PC cells. Abrogation of NRP1/FOXM1/c-Myc signaling axis by small molecule inhibitors or genetic silencing significantly abrogated the recruitment and reprogramming of the indolent PC cells by the aggressive MICs.

Conclusions: MICs are identified in cells isolated from metastatic PC patients and in CTCs from CRPC patients. MICs in clinical PC specimens predict the growth and survival of PC patients. MICs are leader cells that recruit and reprogram indolent bystander cells to express MIC phenotypes and behaviors. MIC-induced reprogramming is mediated by c-Myc and FOXM1 transcriptional activation through NRP1 signaling. Understanding the biology and underlying mechanism of the reprogramming process between MICs and bystander cells could lead to potential development of more effective therapeutic strategies to prevent PC progression and metastasis.

Supported by PCF YI Award 2013