Metastasis-initiating cells (MICs) recruit and reprogram bystander indolent cells to undergo lineage plasticity facilitating prostate cancer progression and metastasis

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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. Phenotypic plasticity of cancer cells, reciprocally regulated by the tumor microenvironment, contributes to lineage plasticity (LP). We observed that human PC cells with MIC phenotype recruit and reprogram naïve bystander indolent epithelial cells to promote lineage switch of indolent PC cells to acquire epithelial-to-mesenchymal transition (EMT), stem, and neuroendocrine (NE) phenotypes facilitating PC progression and metastasis. MICs can also activate bone stromal cells to express tumor-promoting and immunosuppressive cytokines or chemokines to promote aggressive behaviors of PC cells. We discovered among the key transcription factors that c-Myc, FOXM1, E2F3, EZH2, and NF-kB play dominant roles mediating the communication between MICs and bystander epithelial and stromal 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 with MIC phenotype were co-cultured with indolent RWPE-1 or primary-tumor-derived HPE-15 cells and bone stromal HS-5 cells in 3-D suspension culture for several days. Programmed 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 reprogrammed PC cells exhibited LP by EMT, stemness, and NE phenotypes, displayed increased growth, survival and invasive characteristics, and formed tumors in mice. Reprogrammed PC cells also commonly share remarkable amount of genes and pathways activated in genetic mouse PC models with loss of PTEN, RB1, and TP53 that exhibit LP and antiandrogen resistance. MIC-induced reprogramming of indolent PC cells is mediated by activation of c-Myc, FOXM1, E2F3, and EZH2 signaling, leading to epigenetic reprogramming of PC cells. MICs also reprogramed and activated bone stromal cells through activation of NF-kB and SOX2 to promote PC aggressive phenotypes and behaviors *in vitro* and *in vivo.*

Conclusions: MICs are identified in cells isolated from metastatic PC patients. MICs found in clinical PC specimens predict the growth and survival of PC patients. MICs are able to recruit and reprogram indolent bystander epithelial and stromal cells to promote aggressive phenotypes and behaviors and facilitate LP of PC cells. MIC-induced epigenetic reprogramming is mediated by transactivation of c-Myc, FOXM1, E2F3, EZH2, and NF-kB signaling. MIC-induced epigenetic reprogramming significantly converges with genomically-induce 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.

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