

## **C1orf116, a gene of unknown function, inhibits the active migratory phenotype of lethal metastatic cancer cells by promoting an epithelial phenotype**

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While advances in the treatment of localized prostate cancer (PCa) have improved five-year survival to near 100%, metastatic disease remains incurable. Lethal metastases are the end-result of a cancer cell that escapes the primary tumor, travels through the vasculature, and eventually invades and colonizes a secondary site. Thus, in order for a cancer cell to metastasize from the primary tumor, it must *move*. In a critical early event of metastasis, a rare population of cells in the primary tumor undergoes a phenotype switch from a proliferating epithelial cell to gain mesenchymal cell characteristics, including high migratory capacity, during the process of epithelial-to-mesenchymal transition (EMT).

Using a multi-study gene expression discovery analysis, we previously identified C1orf116 as a novel candidate driver of EMT (doi: 10.1186/s12885-017-3413-3). C1orf116 is an unnamed gene of unknown function that remains largely uncharacterized in any disease state, including cancer or EMT. To substantiate its potential role in EMT, we queried the Cancer Cell Line Encyclopedia dataset (GSE36133) and found that C1orf116 expression is associated with classical epithelial markers and inversely associated with mesenchymal markers.

To specifically interrogate the role of C1orf116 in EMT, we overexpressed C1orf116 in otherwise C1orf116<sup>low</sup> PC3 cells (PC3-oe). PC3-oe cells demonstrated distinctive cobblestone epithelial cellular morphology. Correspondingly, PC3-oe cultures showed increased epithelial marker expression (including OVOL1, CDH1, ESRP), indicating that C1orf116 expression was sufficient to induce an epithelial transition.

To determine whether C1orf116 influenced migration, we performed a scratch-closure assay. PC3-oe cultures showed reduced motility compared to control, suggesting that C1orf116 restricts motility. Cellular migration is influenced by many factors, including cell-ECM adhesion, “go vs grow” switching, and cell-cell adhesion. We observed that PC-oe established cell-cell contacts more rapidly than cells in the control cultures (time-lapse photography immediately following culture seeding) and hypothesized that C1orf116 promoted cell-cell adhesion. We found that PC3-oe had increased expression of tight junction complexes (including ZO1, ZO2, ZO3). Likewise, siRNA-mediated knockdown of C1orf116 expression in C42b (C1orf116<sup>high</sup>) cells resulted in decreased expression of tight junction proteins.

These findings demonstrate that C1orf116 is a novel driver of the epithelial phenotype. In particular, C1orf116 expression promotes cell-cell tight junctions and inhibits cell movement, thereby restricting the active emigrant phenotype of potentially lethal metastatic cells.

**Conflicts of Interest:** none

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