The Roles of N-cadherin in Three-dimensional Cancer Cell Invasion

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Summary: The contributions of N-cadherin to the multi-cellular migration of cancer cells through a three-dimensional tissue-like environment are examined.

Cancer accounted for 7.6 million deaths worldwide in 2008, but the number of deaths is expected to surpass 11 million by 2030. Almost 80% of cancer originates from epithelial tissue. The high mortality rate of epithelial derived cancer is partly due to the ability of tumor cells to migrate away from the initial growth site and spread elsewhere (metastasis). As a first step of metastasis, cancer cells are thought to undergo a transformation in which they lose typical epithelial cell-cell junctions and develop an invasive phenotype. Surprisingly, some invasive cancer cells appear as a multi-cellular linear cluster, and often highly aggressive cancer cells up-regulate neural (N)-cadherin cell adhesion protein, but we know very little about the roles of N-cadherin in cancer cell interactions and migration.

To gain a mechanistic understanding of cancer cell invasion, we analyzed the cell adhesion between invasive, transformed epithelial cells in a three-dimensional (3D) collagen matrix. Transformed epithelial cells were transfected with shRNA specific to N-cadherin, and the N-cadherin knockdown cells were transfected with N-cadherin mutants for phenotype rescue experiments.

Using 3D cell invasion assays, transformed epithelial cells formed elongated multi-cellular structures, and migrated as a collective unit (Figure). The individual cells in cell clusters migrated faster and more persistently than single cells in isolation. Calcium depletion by EDTA treatment caused the multi-cellular cluster to dissociate and induced membrane extensions in resulting single cells. Furthermore, depletion of N-cadherin disrupted cell-cell contacts and these N-cadherin deficient cells no longer migrated as a collective unit, suggesting that N-cadherin is required for calcium dependent cell-cell adhesion and multi-cellular invasion. Interestingly, the ectopic expression of the N-cadherin cytoplasmic domain enhanced the formation of membrane extensions in N-cadherin knockdown cells. Our data suggest that the cytoplasmic signaling of N-cadherin induces membrane extensions while the presence of N-cadherin mediated cell junctions may suppress membrane extensions. This unique role of N-cadherin may distinguish the leader cells that form membrane extensions at the free leading edge, apart from the follower cells with N-cadherin junctions that suppress membrane extensions.

Together, our findings suggest that the extracellular and cytoplasmic domains of N-cadherin play an important role in regulating membrane extensions, cell cluster formation, and collective migration, thus N-cadherin up-regulation may be the key requirement for metastasis.

Figure: A. GFP expressing cells migrating within a cluster (white arrow). Time in hours, scale bar 20 μm. B. Migration trajectory of a cell in isolation (S) and in a multi-cellular cluster (C). Scale bar 20 μm. C. Displacement between initial and endpoint position for single cells and cells in a cluster. Bar is average.