Chemotactic Migration of Clustered Central Nervous System Progenitor Cells

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Chemotactic Migration of Clustered Central Nervous System Progenitor Cells

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ABSTRACT

Both CNS derived cancer cells and progenitor cells naturally occur in a clustered morphology. The clustering dynamics as well as the effect of clustering on cell migration to a chemokine have been examined. It has been shown that varied CNS derived cell types including Medulloblastoma derived progenitor cells (MGPCs), Medulloblastoma (MB), and retinal progenitor cells (RPCs) cluster predictably over time in vitro, show increased receptor activity when stimulated with a known chemotactant SDF-1α (CXCL12), and show increased Connexin 43 expression with increased receptor activity when stimulated with SDF-1α. The ability of CNS tumor and progenitor cells to be studied in a clustered morphology allows for the study of unique cell behaviors as they relate to cellular communication in response to chemical stimulation, which represents a new avenue for targeting cancers and increasing the efficacy of RPC injection.

METHODS AND MATERIALS

Standard cell culture in neurobasal medium (NBM) was used to observe clustering over 48 hours in a PC well plate. Immunocytochemistry (ICC) was used to access the presence of CXCR4 and Connexin 43 on the cell surface after stimulation with SDF-1α (100 nM). A microfluidic channel was used to expose cells to 0 to 100 nM SDF-1α gradient over 24 hours, and time-lapse microscopy was used to access migration distance. The system was modeled using the following equations:

\[
\begin{align*}
\text{continuity equation:} & \quad \frac{\partial C}{\partial t} + \nabla \cdot (D \nabla C - v C) = 0 \\
\text{convection-diffusion equation:} & \quad \frac{\partial C}{\partial t} + \nabla \cdot (v C) = 0 \\
\text{motion equation:} & \quad \frac{\partial C}{\partial t} + \nabla \cdot (\nabla C) = 0 \\
\text{hydrostatic equation:} & \quad \nabla p = 0
\end{align*}
\]

An illustration of the µLane channel can be seen in Figure 2, with the corresponding gradient profile in Figure 3.

RESULTS

- Cells showed an increase in CXCR4 surface expression after exposure to SDF-1α.
- Increase in receptor expression did not correlate with cell seeding density
- The clustering behavior of MGPCs in vitro more closely approximates the natural condition for tumor cells in vivo.
- The upregulation of CXCR4 after exposure to SDF indicates increased activity of pathways implicated in cell survival and proliferation (PI3K/Akt/mTOR), and cell migration (RAS/RAF/MEK). The mediation of Connexin 43 in conjunction with SDF-1α has been shown in stromal cells and breast cancer cells, and now CNS derived cells. It may play a critical role in migration as mediated through cell-cell contacts. Results indicate that clusters form at higher cell densities and these clusters exhibit directed chemotaxis.
- The study of MGPC, RPC and MB cluster behavior partially elucidates the mechanistic and biochemical paradigms of cell migration, infiltration and possibly differentiation within the CNS.

DISCUSSION

The ability of CNS tumor and progenitor cells to be studied in a clustered morphology allows for a more accurate appraisal of cell-cell interactions and migration behavior. Upregulation of Connexin 43 via SDF-1α stimulation indicates cell communication, which, along with activation of the RAS/RAF/MEK pathway, may play a crucial role in RPC infiltration, as well as cancer maintenance and proliferation. Approximating the in vivo condition by culturing cell clusters has allowed for the study of unique cell behaviors as they relate to cellular communication in response to chemical stimulation, which represents a new avenue for targeting cancers and increasing the efficacy of RPC injection.

CONCLUSIONS

The clustering behavior of MGPCs in vitro more closely approximates the natural condition for tumor cells in vivo. The upregulation of CXCR4 after exposure to SDF indicates increased activity of pathways implicated in cell survival and proliferation (PI3K/Akt/mTOR), and cell migration (RAS/RAF/MEK). The mediation of Connexin 43 in conjunction with SDF-1α has been shown in stromal cells and breast cancer cells, and now CNS derived cells. It may play a critical role in migration as mediated through cell-cell contacts. Results indicate that clusters form at higher cell densities and these clusters exhibit directed chemotaxis. The study of MGPC, RPC and MB cluster behavior partially elucidates the mechanistic and biochemical paradigms of cell migration, infiltration and possibly differentiation within the CNS.

REFERENCES AND ACKNOWLEDGEMENTS

Future Work

Necessary future work includes downstream studies of the SDF-1α-CXCR4 binding pathway with CNS derived cells. Knockdown models would allow for determination of the specific molecular mechanisms of cluster migration. Other chemokine receptors known to be prevalent in CNS cancers such as EGF and HGF may also be investigated for their impact on clustering behavior.

Figure 1. SDF-1α, CXCR4, binding pathway. With binding and end stage of pathways of interest highlighted.