Report for the Australian and New Zealand Head and Neck Cancer Society Foundation Grant 2021 (Adenoid Cystic Carcinoma Fund)

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Title: A tumour-cell based initiation of immune evasion

Project Details

Description:

Adenoid cystic carcinoma (ACC) is a rare salivary gland cancer most frequently arising in the head and neck region. ACC microenvironment exhibits low immunogenicity, favouring immune evasion. A hook-related protein, Gipie, was found to have roles in inflammation and immune response. However, Gipie is aberrantly expressed in some ACC cells.

Aims of the project

1. Construction of 3D ACC-immune coculture model.
2. Determine activation profile after silencing Gipie.
3. Determine proliferation/apoptosis of ACC cells following Gipie expressions modification in ACC and immune cells.
4. Determine Gipie binding partners and identify the intersection between Gipie interacting proteins and immune cascade.

Results

Presence and absence of Gipie in ACC and normal oral cells confirmed respectively. PBMC, Jurkat, and NK confirmed the presence of Gipie. Confocal imaging of some of the ACC cells look very different from the other cancer cells. They are neither oval nor round. Their shape is difficult to define. 3D stacked transmigration panels of activated immune cells were captured. Showed clear signs of activated cells getting trapped in 3 µm diameter pores, wanting to meet the cancer cells on the other side of the membrane. The activated immune cells/$ACC^{-/-}$ subset showed more cytoskeleton intensity compared to activated immune cells/ACC (unaltered). Ostensibly the number of activated immune cells were more in the Gipie silenced group compared to the unaltered group. $ACC^{-/-}$/immune cells showed more percentage of late apoptotic cells than unaltered ACC. No statistically significant difference of apoptosis noted among $ACC^{-/-}$ vs ACC. $ACC^{-/-}$/immune cells showed more late apoptotic cells compared to A-$253^{-/-}$/immune cells. When Gipie is silenced in immune cells, ACC cells showed highest viability and cell number. Both intracellular FITC conjugated IFNγ and extracellular PE-conjugated NKp30 staining are done. NK-92 cells were already IL-2 activated. Gipie silencing in ACC cells resulted in higher activation of NK-92. Multicolour JK activation flow cytometry showed similar results. Very low expression of Gipie and possibly weak binding of Gipie resulted in lack of target protein achievement. We determined immune cells Gipie binding partners. Proteomics analysis showed marked differences among ACC, A-253, OKF6, NK, NK (activated), JK, and JK (activated) experimental groups. Both phosphokinase and apoptotic arrays helped to elucidate the molecular biological aspect of ACC progression and the role of Gipie in ACC.

Summary and Conclusions

Gipie is present in immune cells and helps in its activation. Gipie is also present in ACC cells. The presence of Gipie in ACC cells definitively reduces the activation of immune cells. The project thus opens a new area of research in the field of clinical oncology.