AB069. NR4A1 agonist CsnB may affect macrophage cells primarily within colorectal tumours in order to reduce pro-inflammatory response

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Background: The link between inflammation and colorectal cancer (CRC) is well established, both in its initiation and progression. Members of the orphan nuclear receptor family 4A (NR4A1–3) are emerging as pivotal inflammatory regulators, however their role in inflammation in CRC has not been investigated extensively. Recent data from our laboratory has demonstrated that the use of cytosporone B (CsnB), an agonist for NR4A1, can suppress tumour-associated inflammation via reduced expression of key inflammatory mediators such as IL-1β and IL-8 (n=33). The exact cells CsnB is affecting to modulate these mediators however remains unknown. Our aim was to examine which cells are secreting the inflammatory mediators attenuated by the addition of CsnB, inferring CsnB sensitive cells within the tumour tissue.

Methods: Tumour tissue (n=12) was obtained from patients undergoing colorectal resection, alongside normal colonic control tissue. The tissue was incubated for 8hrs in cell culture media followed by fixation and sectioning. Macroscopic analysis of tissue was then performed followed by Immunohistochemistry (IHC) analysis for IL-8. Furthermore, we optimised the utility of a flow cytometry procedure using machine homogenization for isolating single cell types for future analysis with CsnB.

Results: Macroscopic analysis of histological sections revealed tumour tissue had a significant inflammatory infiltrate compared to normal tissue. Anti-IL-8 IHC demonstrated staining predominantly within macrophages. This was confirmed using anti-CD68 and overlaying the sections. Isolation of individual cell types using our machine homogenisation was successful displaying 70% cell viability compared to non-machine homogenisation displaying a viability of 10%.

Conclusions: These experiments provide further insight into the site of action of NR4A1 receptor agonist CsnB, highlighting that the macrophage cell within the tumour microenvironment may be a pivotal responsive cell. Ongoing studies are further defining the cell of interest by successfully isolating viable individual cells from the tumour for further experimental analysis.

Keywords: Colorectal cancer (CRC); cytosporone B (CsnB); inflammation; NR4A1

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