Understanding the biological significance of high mannose glycans in terms of ovarian cancer metastasis

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Introduction

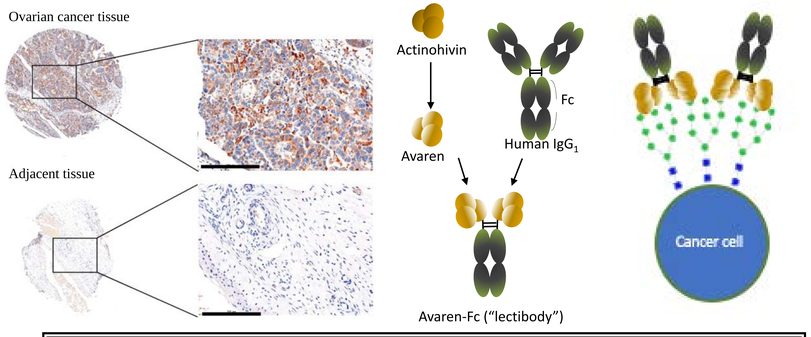
Ovarian cancer is the deadliest gynecological cancer, which begins in the ovaries and eventually metastasizes to the abdomen and other organs. Mortality is attributed to a lack of early detection, a lack of a useful biomarker, and a lack of good second line treatments [1]. Many cancer types, including ovarian cancer, have larger proportions of high mannose N-glycans (HMG) on their surface, which are potentially linked to metastatic activity and which can potentially be used as a biomarker or drug target [2]. Currently, there are no approved diagnostics or therapeutics that make use of this. Our lab has developed a novel plant-made lectin-Fc fusion protein called Avaren Fc (AvFc) that can selectively target these unique biomarkers on the surface of cancer cells and can elicit cell-mediated cytotoxicity [3]. We tested AvFc in four models of human ovarian cancer: the A2780 model, which is a cell line derived from abdominal metastases, and the SKOV3 model, which is a cell line derived from ovarian epithelium. We also tested Av-Fc in two mouse models: 1D8 and the more aggressive ID8 VEGF-DEFB29 (V/D). ID8 is from the mouse ovarian epithelium. ID8 V/D is also derived from the mouse ovarian epithelium and the VEGF portion stimulates the vascularization of the tumor, DEFB 29 promotes growth [4].

1, Jayson, G.C., et al. (2014), The Lancet.
2. Everest-Dass, A.V., et al. (2016), M&CP.
3. Hamorsky, K.T., et al. (2019), Mol. Therapy.
4. Conejo-Garcia. J.R., et al. (2004). Nat. Med.

Objective and Hypotheses

Our goal is to understand whether or not HMGs on the surface of ovarian cancer cells can be used as a biomarker or drug target as well as how these glycans affect metastatic potential. We hypothesize that AvFc can selectively recognize ovarian cancer cells and elicit ADCC, but will not be cytotoxic due to binding alone.

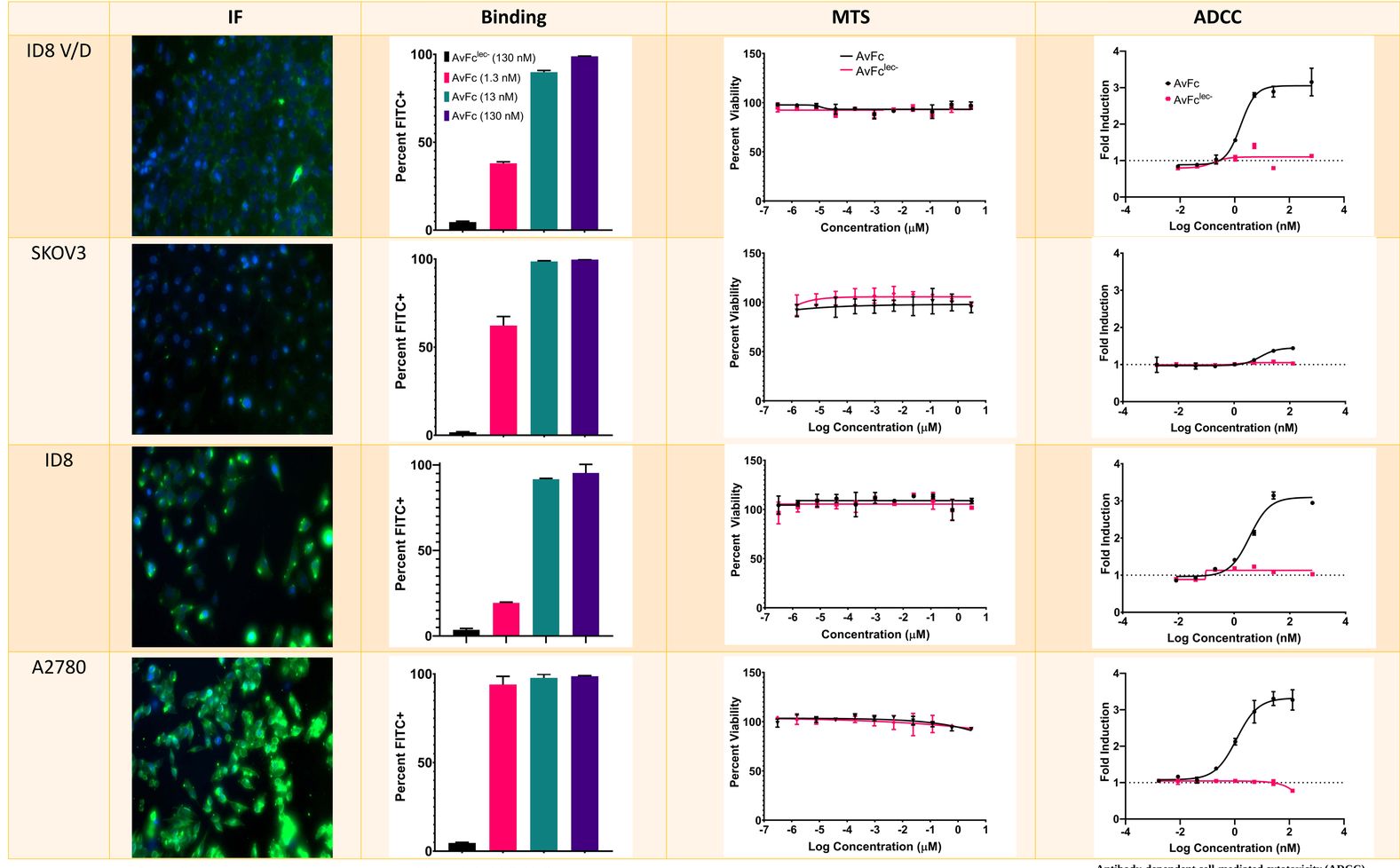
Construction of Avaren-Fc



Methods

1. Immunofluorescence (IF): used to visually demonstrate binding between AvFc and ovarian cancer cells.
2. Flow Cytometry: used to evaluate recognition to ovarian cancer cell lines MTS assay: used to determine cytotoxicity of AvFc due to cell binding.
3. ADCC: used to assess Fc-mediated cytotoxicity by the immune system.

Results



Immunofluorescence of ovarian cancer cell lines. Cells were stained with 130 nM AvFc and a goat anti-human Fc-FITC, and counterstained with DAPI. AvFc bound strongest to A2780 cells, corresponding with the flow cytometry data.

Flow analysis of AvFc binding to ovarian cancer cells. Cells were stained with 1.3, 13, and 130 nM of AvFc and 130 nM AvFc lec-, a non-sugar binding mutant. AvFc binds strongly to each cell line, with saturation occurring at roughly 13 nM.

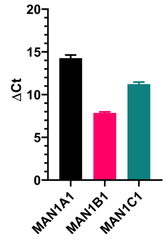
Assessment of cell viability after AvFc exposure with MTS reagent. Cells were exposed to AvFc or AvFc lec- beginning at 3 μM with 1:5 serial dilutions for 48 hours. Cell viability was then assessed 4 hours after adding MTS reagent. AvFc showed no cytotoxicity or growth inhibition in any of the lines tested.

Antibody-dependent cell-mediated cytotoxicity (ADCC) activity of AvFc. Cells were incubated with AvFc or AvFc lec- beginning at 650 nM, with 1:5 serial dilutions. After the addition of 150,000 Jurkat-FcγRIIIa-luc effector cells, plates were incubated for 24 hours at 37°C and luminescence was measured after the addition of a luciferase reagent. AvFc elicited a 3x increase in luminescence against A2780, ID8, and ID8 V/D cells but did not show significant effect against SKOV3 cells.

Conclusions and Future Directions

- AvFc binds strongly to all 4 of the ovarian cancer cell lines.
- AvFc is not directly cytotoxic and does not inhibit growth, but can interact with the immune system and potently induce ADCC against cancer cells.
- **These data agree indicate that the mechanism of action of AvFc is mostly immune-mediated.**
- Future studies will be done to evaluate efficacy of AvFc using *in vivo* ovarian cancer models and the effects of HMGs on ovarian cancer metastasis

Mannosidase Expression in A2780 Cells



Mannosidases trim HMGs, and may be deficient in some cancer cells, resulting in increased high mannose glycans on the surface. Overexpression of mannosidase 1C1 can decrease high mannose glycans on the surface, which potentially could affect ovarian cancer metastasis.

Acknowledgements

- University of Louisville Cancer Education Program NIH/NCI (R25-CA134283)
- NIH grants (R21CA216447
- Dr. Steve Fiering of Dartmouth University and Dr. Karen Mclean of the University of Michigan for providing ID8 cells.