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The Spriggs laboratory has been focused on proteins present on the ovarian cancer cell surface and how those proteins regulate function in health and cancer. In particular, we are interested in MUC16 and Galectin 3. Our studies over the past several years have provided insights into the function of MUC16. It is now apparent that the MUC16 regulates functions like cancer growth and spreads through changes in the structure of sugars (glycosylation) on the surface of cancer cells. This regulation requires interaction with specialized sugar binding proteins called Galectins, which are key components of the tumor microenvironment. We are actively developing new antibodies against MUC16 and Galectin 3 for diagnosis, imaging and treatments. Our work has shown that antibodies which inhibit these cell – cell interactions can slow tumor growth and block the spread of cancer cells locally and inhibit the spread to new organs.

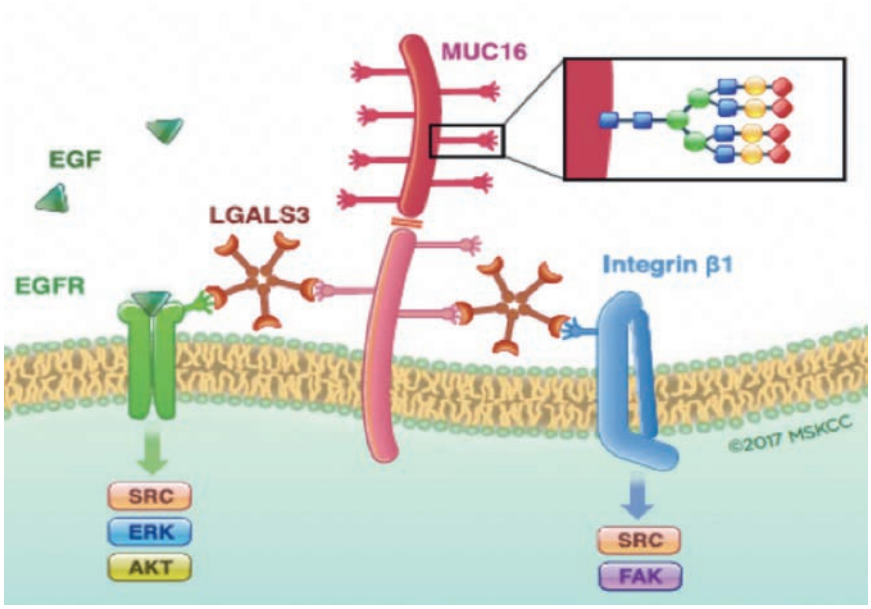
Our research group is actively examining the role of glycosylation, especially on mucins in tumor specific behaviors including uncontrolled growth, oncogene activation, invasion, immune system evasion angiogenesis, and metastatic spread. This work includes potential therapeutic antibodies against MUC16 and Galectin-3 in cancer.

Anti-MUC16 biology

Our current MUC16 work concentrates on development of our human MUC16 antibodies for targeting ovarian cancer. Our antibodies uniquely target the most proximal, retained portion of the MUC16 following cleavage and release of the CA125 antigen into the circulation. This retained ectodomain is a 58 amino acid peptide, linked to the membrane via a short transmembrane domain and a 31 amino acid cytoplasmic tail which is linked to the cellular cytoskeleton for mobility. We have shown that most of the adverse consequences relate to MUC16 expression. As little as 114 amino acids from the carboxyl terminal of the intact MUC16 sequence is sufficient to promote increased soft agar colony formation, Matrigel invasion with increased MMP2/MMP9 expression, activation of both AKT and ERK proto-oncogenes, and enhanced growth in nude mice. Deletion experiments demonstrate that the 58 amino acid MUC16 ectodomain is required for this effect. If one examines the ectodomain in greater detail, the portion of the sequence containing 2 N-glycosylation sites is the essential element. We (esp. Dr. Lee) are now actively examining the structure of the MUC16 – antibody interaction to improve the therapeutic efficacy of antibodies.

MUC16-directed Chimeric Antigen Receptor (CAR) T Cells

Chimeric Antigen Receptor (CAR) T cells have not been successful in the management of solid tumor malignancies. Reasons for this include: poor trafficking, the presence of an immunosuppressive tumor microenvironment, CAR T-cell dysfunction and immune escape via antigen-loss. In conjunction with Dr. Oladapo Yeku, from our junior faculty, we are using our antibodies as MUC16 targeted CAR T cells. We are developing strategies to further modify CAR



MUC16 is an example N-glycosylation - rich molecule which can regulate the cellular location and signal transduction mediated by TK receptors like EGFr or adhesion molecules (integrins) through Galectin 3 mediated interactions.

T cells to optimize their efficacy for ovarian cancer and gynecologic malignancies. Our approaches to further engineering these CAR T cells with Human Artificial Chromosomes (Dr. Kononenko) are informed by the ovarian cancer tumor microenvironment. Using syngeneic immune competent mouse models and subsequent validation in genetically engineered and xenograft models, we are able to effectively evaluate these rationally optimized CAR T cells as monotherapy or in combination with other immunomodulatory agents prior to initiation of clinical trials.

Glycosylation Dependence

Our work has been the first to show that the oncogenic effects of MUC16 require MGAT5 dependent tetra-antennary glycosylation of the MUC16 ectodomain and interaction with Galactin 3 (LGALS3). This complex then binds to glycosylation sites on growth factors including EGFr, Integrins, and immune receptors like CTLA4. This has provided us with new opportunities for MUC16+ cancer cell targeting.

Galectin 3 Targeting

LGALS3 regulates the interaction of surface proteins with the extracellular membrane domain and mediates a signal cascade leading to invasion, oncogene activation and growth. While anti-MUC16 glycosylation site antibodies inhibit oncogenic properties, LGALS3 represents a more general strategy for targeting glycosylation dependent oncogenesis. We have developed high-affinity antigalectin-3 antibodies directed at the carbohydrate recognition domain (CRD) of the galectin-3 carboxyl-terminus (to block sugar binding). These antibodies are able to block the oncogenic effects of MUC16 expression including invasion, oncogene activation (AKT, ERK, SRC) and reduced growth in nude mice. In addition, these antibodies appear able to decrease metastatic behaviors in lung metastasis models. Dr. Xu is focused on the functions of Galectin 3 in cancer while Dr. Lee has been producing a structural model of binding to the Galectin-3 surgery binding elements.

Selected Publications:

Yeku OO, Purdon TJ, Koneru M, **Spriggs D**, Brentjens RJ. Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment. *Sci Rep*. 2017 Sep 5;7(1):10541.

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Dharma Rao T, Park KJ, Smith-Jones P, Iasonos A, Linkov I, Soslow RA, **Spriggs DR**. Novel monoclonal antibodies against the proximal (carboxy-terminal) portions of MUC16. *Appl Immunohistochem Mol Morphol*. 2010 Oct;18(5):462-72.

Zhao Y, Cao J, Melamed A, et al. Losartan treatment enhances chemotherapy efficacy and reduces ascites in ovarian cancer models by normalizing the tumor stroma. *Proc Natl Acad Sci USA* 2019:pii:201818357. 10.1073/pnas.1818357116.