ABSTRACT

The glycosylation pattern of plasma-membrane resident proteins plays a pivotal role in interaction amongst the cells and between the cells and the extracellular matrix. A dramatic change in glycosylation of membrane receptors is one of the hallmarks of cancer. One particular glycol-signature which is significantly altered but less-explored is the sialylation of membrane receptors & glycoproteins. Change in the sialome of membrane receptors and glycolipids has been associated with cell migration & tumor advancement; however, the direct link is extremely poorly understood. Increased activity of plasma membrane-localized sialidases in cancer could act as a trigger for rapid endocytosis of desialylated membrane cargoes with the help of receptor binding proteins from the ECM like galectins. The current hypothesis is that the desialylated cargoes could be transported to the Golgi for resialylation and secreted in a polarized manner, leading to persistent cell migration & hence enhanced disease progression. *(Ref: Shafaq-Zadah, M., Gomes-Santos, C., Bardin, S. et al.)*

Hence we ask the question, "What regulates the retrograde transport of membrane proteins at the plasma membrane?" To explore this, we focus mainly on one membrane protein – Epidermal Growth Factor Receptor. We think that receptor glycosylation, particularly the sialylation of receptors, plays an essential role in regulating the receptors' retrograde transport. Hence, we try to nail down this mechanism to molecular details to dissect the machinery involved at the plasma membrane that triggers the activation of sialidases.

The next point that we want to understand is, "What regulates receptor sialylation?" We hypothesize that sialidases' activity plays a significant role in the sialic acid pattern on the plasma membrane proteins and lipids. In humans, there are four sialidases or neuraminidases – Neu1, Neu2, Neu3, and Neu4. siRNA screening experiments have proved that Neu1 and Neu3 are of particular interest in the effect of EGF towards cell migration in cancer. These two neuraminidases are highly localized in the plasma membrane of cells. In a normal cell, when EGF binds to the EGF receptor, it causes the receptor's desialylation. The desialylation stimulates the neuraminidases, which act as a scissor, chopping off sialic acid decorations from the nearby membrane proteins. Through their exposed galactose sites, these desialylated proteins are bound by galectins present in the extracellular matrix. Galectin binding plasma membrane protein and their subsequent oligomerization provide a push force from the outside to form endocytic pits, marking the beginning of clathrin-independent endocytosis through the

GL-Lect pathway of retrograde endocytosis. The proteins, following the retrograde endocytosis, are returned to the plasma membrane, however, in a polarised manner. This process gets up-regulated in cancer cells, where we see an activation of the neuraminidases even in the absence of EGF ligand. It is believed that the polarisation of the cells gives directionality and hence aids metastasis. Thus, we want to see the physiological effect of down-regulating the neuraminidases, especially Neu1 and Neu3, in the cancer cells. The level of sialylation at the plasma membrane will be checked beforehand using galectin binding, where we apply our knowledge that galectin 3 binds to the desialylated membrane proteins, whereas galectin 8 binds to the sialylated membrane proteins. This experiment will also give us an idea if the sialylation pattern of the membrane proteins can be altered using externally provided sialic acid.

The differential galectin binding to the cell surface was quantified earlier, which gives us an idea of the sialylation pattern of the plasma membrane receptor proteins.

Once the galectin binding pattern, and hence the sialylation pattern of the membrane proteins, is determined, we plan to investigate next the enhanced cell migration pattern in 3D spheroids of MDA-MB-231 cells. We ask ourselves the question, "How does sialylation of plasma membrane proteins affect the cell migration pattern in a tumor." We use a 3D model because it represents a better fit to the in vitro system than a monolayer 2D cell model. We use a small molecule inhibitor of neuraminidase, DANA, to determine the effect of down-regulation of the plasma membrane neuraminidases on cell proliferation and metastasis.