Decoding Cancer Glycosylation Through Single-Cell Multiomics

Glycosylation is one of the major post-translational modifications in humans and is defined by the attachment of glycans to lipids and proteins *via* a multilayered enzymatic reaction (**1**). For proteins, O-linked and N-linked glycans account for most of the glycan modifications (**Fig. 1**) (**2**). Glycosylation is involved in the regulation of essential processes such as cell migration, immune recognition, receptor-ligand interactions, and regulating protein activity (**1**, **3**). Thus, dysregulated glycosylation is a hallmark of various cancer entities mainly by triggering pro-tumorigenic processes such as cancer cell survival, angiogenesis, and inflammation (**3**).

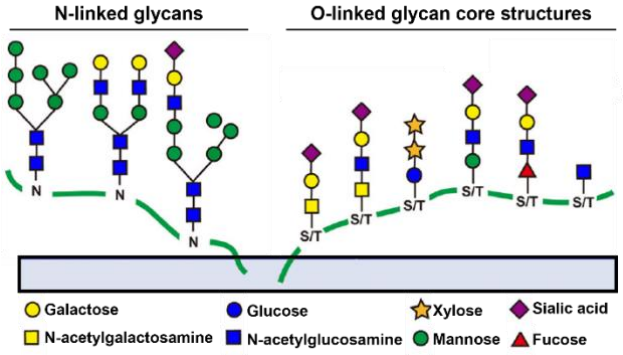

Figure 1. Overview of N- and O-glycans observed in cancer tissues (image modified from Guo et al. (1).

Abnormal glycosylation is described to be associated with cancer development, disease progression and metastasis, and can be used as a prognostic biomarker as tumor cells often display elevated protein glycosylation (**4**). Under steady-state conditions, glycans control important cellular pathways involved in cell death and survival signaling (**5**). Yet, in tumor tissues, aberrant expression of molecules involved in glycosylation or metabolic changes can lead to altered glycosylation of cell surface proteins and therefore, a disruption of these signaling cascades eventually promotes pro-tumorigenic processes (**5**, **6**).

Tumor cells are known for using aerobic glycolysis for energy supply. The shift in energy source is characterized by higher glucose and glutamine uptake and influenced by hexosamine biosynthetic pathway (HBP) that results in the synthesis of UDP-GlcNAc which is later used in both N- and O-glycosylation. Moreover, it has been implicated that hyper O-GlcNAcylation is associated with many cancer types including lung, prostate, liver, and colorectal cancers. (**7-8**). Yi et al. showed that in addition to its role as a nutritional sensor, O-GlcNAc glycosylation can directly regulate glycolysis and alter the metabolism in cancer cells *via* phosphofructokinase 1 (PFK1) which involves production of metabolites for rapid cell growth (**9**).

Besides the malignant transformation leading to excessive cancerogenic proliferation, another important hallmark of cancer cells is the ability to circumvent apoptosis, a form of programmed cell death (**10**). Apoptosis can be conducted *via* an intrinsic, mitochondria-dependent or an extrinsic, death receptor-mediated pathway, where glycosylation was reported to regulate various intra- and extracellular factors, pathways and mechanisms involved in these signaling cascades. Not surprisingly, changes in glycosylation have been

reported in several human cancer entities (**11**). The death receptors Fas, TRAIL-R1/2 and TNFR1 can induce apoptotic cell death upon activation but various studies have shown that aberrant glycosylation can affect signal transduction, internalization, and complex formation leading to dysregulated cell death signaling which could impact cancer cell survival and tumorigenesis (**11-14**). Additionally, α2-6-linked sialylation mediated by the glycosyltransferase ST6Gal-I was described to modulate the susceptibility towards pro-cell death signaling and thereby influence cancer pathogenesis (**13, 14**).

Although the implication of altered glycosylation in the context of cancer pathogenesis is well established and is used for diagnostic and therapeutic approaches, the underlying mechanisms and pro-tumorigenic effects of dysregulated glycosylation are still not fully understood (**2**, **6**). Up to now, only limited information on the tightly regulated interplay between cell surface glycans and cancer pathogenesis is available on a single-cell level.

The immune cells in tumor microenvironments are also subject to regulation by glycosylation. In a recent study, the group of Dr. Jie Li from Nanjing University developed a single cell multi-omics analysis method that apples LacNAc labeling to analyze the glycosylation levels of CD8 T cells isolated from a murine breast cancer model. With this method, the authors showed that glycosylation was enhanced in effector CD8 T cells whereas levels were lower in other CD8 T cell subsets, including exhausted CD8 T cells (**Fig. 2**) (**15**).

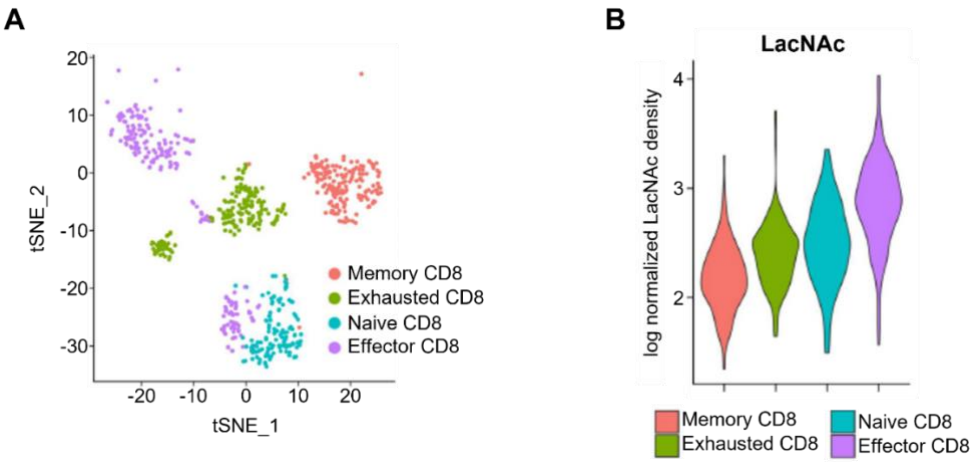

Figure 2. Glycosylation levels in CD8 T cells isolated from a murine breast cancer model.

(**A**) tSNE blot from tumor infiltrating CD8 T cell subsets. (**B**) Violin blot from LacNAc levels from isolated CD8 T cells (image modified from Yu et al. (**15**)).

In collaboration with Dr. Li's group, Singleron developed ProMoSCOPE™ technology to gain more insights into the role of glycosylation in the development and progression of tumors on a single-cell level. The technology allows for chemoenzymatic labeling of the N-acetyllactosamines (LacNAc), commonly found in cell surface glycans, using a dedicated ProMoSCOPE™-tag (Figure 3A). Combined with Singleron's SCOPE-chip® technology for high-throughput single-cell transcriptome profiling, ProMoSCOPE™ enables the simultaneous quantification of glycosylation levels together with a complete gene expression profile within the same single cells (Figure 3B).

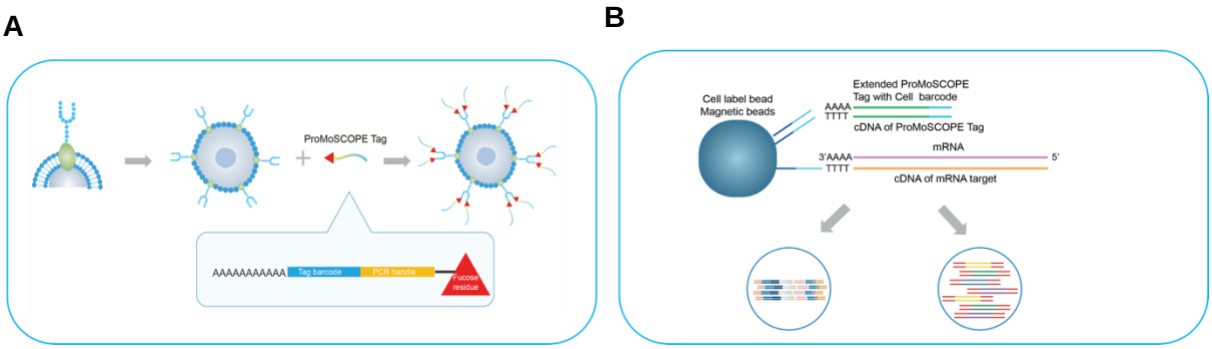

Figure 3. Schematic representation showing the principle of ProMoSCOPE™ technology.
Cells are chemoenzymatically labeled with ProMoSCOPE Tag that contains barcode and poly A sequences. ProMoSCOPE Tag specifically recognizes N-acetyllactosamine (LacNAc) at the cell surface (**A)**. Upon cell lysis, barcoding beads capture both ProMoSCOPE Tags and mRNA by poly-A tails and two libraries are prepared that can be sequenced together **(B)**.

Various immune cells participate in the immune response against cancer. These cells can detect and target cancer cells by recognizing specific molecules on their surface, known as antigens. However, cancer cells can develop various strategies to evade the immune system. They may alter or downregulate the expression of antigens, impair immune cell recognition, or create an immunosuppressive microenvironment within tumors. This immune evasion allows cancer cells to proliferate and spread. Quantifying cell surface glycosylation, with Singleron's ProMoSCOPE™ kit, at the single cell level has the potential to advance our understanding of the mechanisms of cancer development and metastasis. In the experiment below, ProMoSCOPE technology has revealed different glycosylation abundance in the bone marrow (Figure 4). Neutrophils and macrophages displayed higher levels of glycosylation.

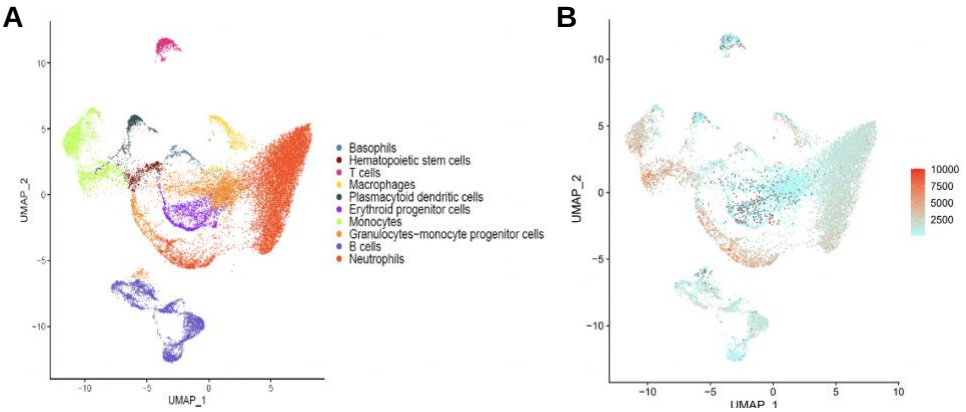

Figure 4. ProMoSCOPE ™ reveals heterogeneity of glycosylation abundance in the bone marrow.
Mouse bone marrow is extracted and libraries are prepared with ProMoSCOPE Single Cell Glycosylation Detection Kit. Major cell types are identified in the sample (A). UMAP displaying the tag UMI counts to indicate the levels of glycosylation for each cell type (B).

To learn more about the **ProMoSCOPE™** technology and other single-cell multi-omic approaches Singleron offers, visit our website at **www.singleron.bio** or email us at **info@singleronbio.com** and get in touch with one of our single-cell specialists to discuss how we can further your research.

Product	Tissue
	2 RXNs / 16 RXNs
ProMoSCOPE™ Single Cell Glycosylation Detection Kit Tissue	1251011 /1251012
ProMoSCOPE™ Single Cell Glycosylation Detection Kit Tissue for Matrix	1251021/1251022

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