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ABBIE, A GUIDED INTEGRASE, ESTABLISHES STABLE SKOV3-GYS1 CELLS FOR METABOLIC DRUG SCREENING

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ABSTRACT

Glycogen synthase 1 (GYS1) is a key enzyme in glycogen metabolism implicated in cancer cell survival and therapy resistance. Elevated GYS1 expression correlates with aggressive disease and poor prognosis in several cancers, including triple-negative breast cancer, highlighting glycogen metabolism as a targetable metabolic vulnerability in tumors. Here we report the generation of a GYS1-overexpressing ovarian cancer cell line (SKOV3-GYS1) using ABBIE (A Binding-Based Integrase Enzyme), a novel gene integration platform based on an HIV-1 integrase fused to dCas9. Unlike conventional CRISPR–Cas9 systems, ABBIE directs donor DNA insertion without introducing double-strand breaks or requiring viral vectors. A donor construct encoding the human GYS1 coding sequence and a neomycin resistance gene were co-delivered with ABBIE mRNA and sgRNAs. sgRNA design was based on conventional CRISPR design and identification of landing site was assessed via whole genome sequencing. Following G418 selection, bulk populations exhibited stable donor integration, and ELISA confirmed ~3-fold higher GYS1 protein expression relative to parental SKOV3 cells, while GYS1 catalytic activity was estimated to be over 5 times higher than that of parental, non-transduced cells. Whole-genome sequencing of the highest-expressing clone revealed a unique single integration event at an intergenic locus. This property establishes ABBIE as a system that is targetable in a reproducible and non-random fashion, via a distinct mechanism currently under investigation. The resulting SKOV3-GYS1 line provides a robust in vitro platform for studying glycogen metabolism in ovarian cancer and for screening novel GYS1 inhibitors. The introduction of one copy number with no detectable secondary integration sites results in a powerful biological tool for drug discovery.

BACKGROUND

Tumor cells often reprogram their metabolism to survive under stress conditions such as hypoxia and nutrient deprivation. Glycogen, a branched glucose polymer, has emerged as an important energy reservoir in cancers[1]. Once thought to be merely a glucose storage depot, glycogen is now known to lie at the nexus of processes that promote malignancy, including cancer cell proliferation, invasion, and chemoresistance. Many tumors exhibit dysregulation of glycogen metabolic enzymes – for example, aberrant expression of glycogen synthase 1 (GYS1) has been documented in cancers of the kidney, ovary, lung, liver, blood, and breast. In breast cancers, high GYS1 expression is associated with significantly worse patient survival, especially in highly proliferative subsets like triple-negative breast cancer[2]. Conversely, experimental knockdown of GYS1 can impair cancer cell growth, deplete glycogen stores, and increase sensitivity to metabolic stress. These findings highlight GYS1 as a potential therapeutic target in oncology. Targeting glycogen metabolism in cancer is a promising yet under-explored strategy, and there is a growing interest in discovering small-molecule GYS1 inhibitors or other glycogen-modulating drugs.

To facilitate the development and testing of glycogen-targeted therapies, robust cellular models with defined alterations in glycogen metabolism are needed. We focused on creating an ovarian cancer cell line with stable overexpression of GYS1. Ovarian cancer remains a deadly disease where metabolic adaptation can contribute to therapy resistance; for instance, glycogen accumulation (mediated by GYS1) has been implicated in the chemoresistance of certain ovarian tumor subtypes.[1] We chose the SKOV3 cell line, a widely used human ovarian carcinoma line, as the base for engineering a GYS1-overexpressing model. Such a model can be used to screen candidate GYS1 inhibitors and to investigate how excess glycogen synthesis affects tumor cell behavior *in vitro.*

Here, we employ an alternative genome editing approach that does not rely on cutting the host DNA: the ABBIE platform. ABBIE (A Binding-Based Integrase Enzyme) is a fusion of a catalytically inactive Cas9 (dCas9) with a retroviral integrase, which together enable guide RNA-directed integration of a donor DNA sequence into the genome. The integrase component derives from HIV-1, providing the machinery for DNA insertion, while the dCas9 domain provides targeting by binding to a user-specified genomic sequence without cleaving it. Importantly, the ABBIE method avoids the use of viral vectors and does not trigger DNA damage response pathways as happens with Cas9, since no double-strand breaks are made. It is also capable of integrating relatively large DNA payloads (>7 kb) in a single step, which is advantageous for inserting complete gene cassettes. We hypothesized that ABBIE could achieve a knock-in of the GYS1 transgene into SKOV3 cells with high efficiency and potentially fewer off-target events compared to conventional methods.

FINDINGS

In this study, we applied the ABBIE system to generate a GYS1-overexpressing SKOV3 cell line. We designed a donor DNA construct containing the full-length human GYS1 coding sequence along with a selectable marker for neomycin resistance. This donor was co-delivered with in vitro-transcribed ABBIE mRNA (encoding the dCas9–HIV integrase fusion protein) and gene-specific single-guide RNAs. sgRNA design was based on conventional CRISPR design using publicly available bioinformatic tools.

Following transfection and antibiotic selection (G418), we obtained a polyclonal cell population. The bulk population was cloned by limiting dilution and five clones were obtained. This group of clones was tested by ELISA-based GYS1 expression analysis.

GYS1 Protein Expression Analysis

To quantify GYS1 protein expression in the five clonal isolates with fastest-growing characteristics, we used the Human Glycogen Synthase 1 SimpleStep ELISA® kit (Abcam, ab214031).
Cell pellets from each clone were lysed in chilled 1X Cell Extraction Buffer PTR, clarified by centrifugation, and diluted to obtain a total of 100 ug lysate/well. Optical density was measured at 450 nm using a plate reader with background subtraction. All five clones demonstrated elevated GYS1 protein relative to unmodified cells, with Clone 3-1 showing the highest increase, ~3-fold over parental levels.

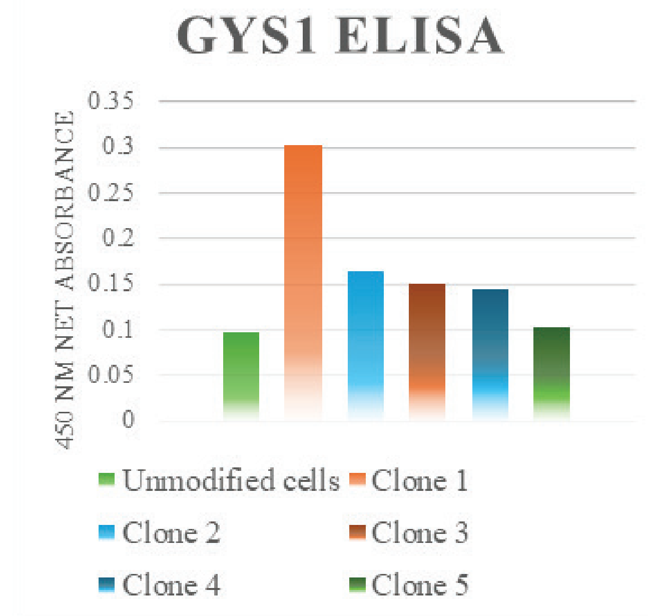

Figure 1. **Quantification of GYS1 protein expression in SKOV3 parental cells and ABBIE-modified clones.** GYS1 protein levels were measured in lysates from unmodified SKOV3 cells and five clonal isolates derived from the fastest-growing ABBIE-edited population using the Human Glycogen Synthase 1 SimpleStep ELISA® kit (Abcam, ab214031). Values represent mean net absorbance at 450 nm after subtraction of blank control wells. All five clones demonstrated elevated GYS1 protein relative to unmodified cells, with Clone 3-1 showing the highest increase (~3-fold over parental levels).

Integration Site Analysis

Targeted-capture NGS analysis of the DNA derived from the bulk population revealed a dominant integration site, occurring within a transcriptionally inactive intergenic region on chromosome 1, with no known active genes. Specifically, 80.5% of total integration events were mapped on a single location on chromosome 1, 14.8% on another single location on chromosome 8, also devoid of known genes, 3.5% on chromosome 7, and 1.2% on chromosome 6.

When we further sequenced the DNA of clone 3-1, it was discovered that the integration locus was unique. Conventional retroviral and lentiviral vectors typically integrate at multiple semi-random sites across the genome, producing a heterogeneous insertion pattern that complicates both safety assessment and long-term monitoring. In contrast, ABBIE editing generated a single dominant locus that could be fully resolved. The ability to guide integration events with such precision at a single genomic location enhances the regulatory profile of ABBIE, as it allows for rigorous genomic characterization, facilitates comparability across clones, and provides a clearer path toward safety evaluation relative to traditional viral approaches.

In summary, whole-genome sequencing of clone 3-1 revealed that the donor cassette was present as a single integration event. This finding indicates that the majority of cells within the originating bulk population had received the donor DNA at one genomic locus, rather than at dispersed sites. The uniform single-site integration observed in 3-1 demonstrates ABBIE's potential to generate clonal populations with consistent genomic architecture, a property that could improve both experimental reproducibility and safety profiles relative to traditional viral methods.

These observations suggest that ABBIE is targetable, though not by conventional Cas9 guide design rules. In our experiments, the gRNA designed according to conventional CRISPR/Cas9 rules did not correlate to identified ABBIE insertion sites. The fact that a dominant integration site emerged under this condition implies that ABBIE can be guided in a consistent and non-random manner, even if the exact insertion coordinates diverge from the canonical CRISPR–Cas9 target.

While additional experiments will be needed to establish reproducibility across replicates and cell types, the singular nature of the observed insertion strongly suggests that ABBIE can be steered toward defined genomic addresses, supporting the concept of a predictable, albeit mechanistically distinct, targeting process.

Several factors could underlie the discrepancy between the intended target and actual integration site. One possibility is that the dCas9-integrase fusion may tether the pre-integration complex to the vicinity of the guide RNA binding site, but the integrase then inserts the DNA at the nearest acceptable chromatin location that meets its sequence or structural preferences. Indeed, HIV integrase is known to favor certain DNA sequence motifs and structural features, such as bending flexibility, at the insertion site[3,4]. If the guide RNA target region lacks an optimal insertion site, or if it is in a less accessible chromatin context, the integrase might settle for a nearby locus that is more integration-permissive. Another contributing factor could be the mutant ABBIE version used in this validation experiment. While this mutation was intended to disrupt integrase's interaction with host tethering factors (like LEDGF) and thereby reduce bias towards canonical HIV integration hotspots[5], it might also have introduced a new bias or preference. Prior studies of integrase retargeting have shown that single amino acid changes can dramatically alter integration site selection[5]. It is conceivable that the current mutant ABBIE causes the integrase to favor genomic features that differ from LEDGF-directed sites.

It is important to note that although we did not achieve insertion precisely at the conventional CRISPR designed target , the outcome still met our primary goal: generating clones with stable, high GYS1 expression. For the purpose of an in vitro drug screening tool, the exact genomic location of the transgene may be less critical, provided that the expression is consistent and the integration does not disrupt any essential genes. In our case, the clones have been growing robustly with no obvious fitness issues, implying that the off-target integrations did not hit any lethal gene. However, ensuring truly site-specific integration will be important for certain applications (e.g., therapeutic cell engineering or precise genotype-phenotype studies). The discrepancy observed here highlights that ABBIE, in its current form, will require further optimization for targeting fidelity, which is the object of a current study.

Current and future studies will explore strategies to improve the specificity of ABBIE-mediated integration. One approach is to refine guide RNA design: for instance, selecting guide sites that coincide with sequences known to be favored by HIV integrase, or using multiple guide RNAs simultaneously to tether the integrase complex to a region from several angles. Another approach is protein engineering – for example, modifying the fusion protein linker to better constrain the integrase's action radius. Notably, aside from the targeting unexpected result, the efficiency of integration we observed was quite high. Virtually all G418-resistant colonies we screened contained the donor, as evidenced by ELISA results in clonal isolates. This suggests that ABBIE successfully integrated the donor DNA in a large fraction of cells when the full system (donor +integrase + sgRNA) was delivered.

Western Blotting

To confirm ABBIE-mediated GYS1 overexpression at the protein level, we performed Western blot analysis on lysates from ABBIE-edited SKOV3 clones and parental SKOV3 cells. Clones derived from ABBIE integration exhibited a clear band at the expected molecular weight for GYS1 (~85 kDa), with notably higher intensity relative to the unmodified parental control. The signal was consistent with the ~3-fold increase previously observed by ELISA, indicating that the ABBIE-mediated integration resulted in the production of full-length, correctly sized GYS1 protein. Importantly, β-actin loading controls demonstrated comparable protein input across all samples, supporting that the observed differences reflect true increases in GYS1 abundance rather than loading variation. Together, these results validate ABBIE's ability to generate stable, high-level GYS1 expression in ovarian cancer cells.

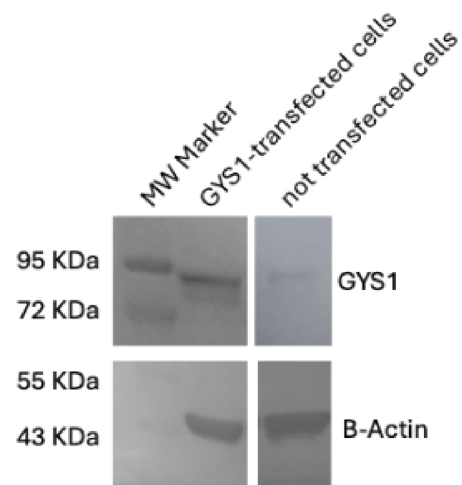

Figure 2. **Western blot confirmation of GYS1 overexpression in ABBIE-modified SKOV3 cells.** Whole-cell lysates from parental SKOV3 cells and ABBIE-edited clones were resolved by SDS–PAGE and probed with anti-GYS1 antibody. A prominent band corresponding to full-length GYS1 (~85 kDa) was detected in ABBIE-modified clones, with higher intensity compared to the parental line. β-actin served as a loading control and confirmed equal protein loading. These results corroborate ELISA-based quantification, demonstrating that ABBIE integration yields robust and correctly sized GYS1 protein expression.

GYS1 Activity

Glycogen synthase activity was monitored over 600 s by following NADH consumption at 340 nm in a PK/LDH-coupled assay as previously described[6]. Linear regression across the entire measurement window yielded slopes of –0.0176 ΔA340/min (R = –0.65) for non-transduced cells and –0.0797 ΔA340/min (R = –0.99) for GYS1-transduced cells. These correspond to reaction rates of 5.65 μM/min and 25.6 μM/min NADH consumption per well, respectively. After normalization to protein content in the cell lysates (5.97 mg/mL for controls, 5.18 mg/mL for transduced cells), the calculated specific activities were 0.95 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ and 4.94 $\mu mol \cdot min^{-1} \cdot mg^{-1}$, respectively.

This represents a ~5.2-fold increase in glycogen synthase activity upon GYS1 overexpression, confirming that recombinant GYS1 expression substantially enhances enzymatic function compared to baseline.

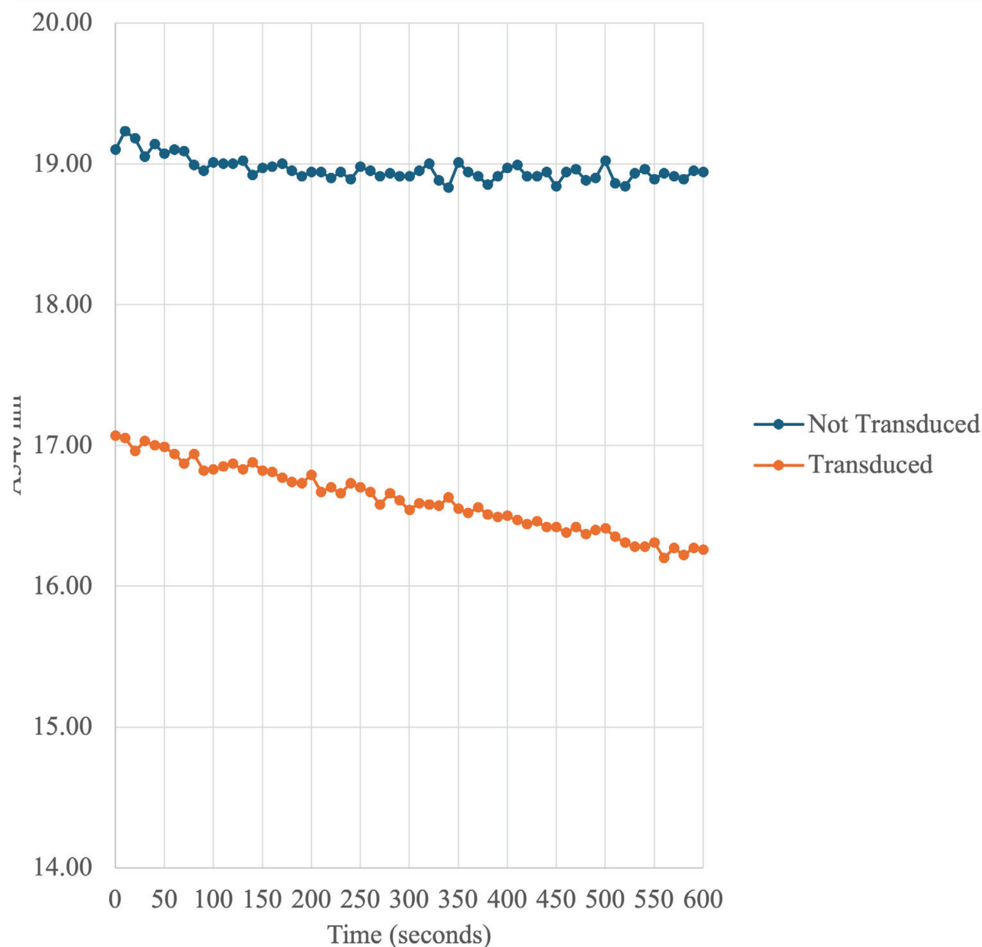

Figure 3. **Glycogen synthase activity assay in non-transduced and GYS1-transduced cells.** NADH consumption was monitored spectrophotometrically at 340 nm over 600 s using a pyruvate kinase/lactate dehydrogenase-coupled assay.

DISCUSSION

To our knowledge, this is the first report of deploying a catalytically inactive Cas9-guided integrase to knock a metabolic gene into the cancer cell genome. The engineered SKOV3-GYS1 cells showed robust overexpression of GYS1 protein, approximately three-fold higher than the parental line by ELISA, indicating that the inserted transgene is functionally active and that the chosen promoter/enhancer elements in the donor are driving high expression. This level of overexpression is expected to significantly elevate intracellular glycogen synthesis, which will be verified in future experiments by measuring glycogen content and metabolic flux in the clones.

From a practical perspective, the SKOV3-GYS1 cell line developed in this work is a valuable addition to metabolic cancer research tools. We demonstrated that this cell line stably expresses elevated GYS1 levels through at least an initial expansion. We also performed Western blot analysis and confirmed that the overexpressed GYS1 is the correct molecular weight, and we were able to correlate the ELISA-based quantitation with Western blot protein band intensity and biological activity in the over-expressing clone compared with the unmodified parental cell line. This SKOV3-GYS1 line can be used to screen for small molecules that inhibit GYS1 or that are selectively lethal to cells with hyperactivated glycogen synthesis. This model can also help explore biological questions such as how excess glycogen storage affects ovarian cancer cell signaling, growth in hypoxic conditions, or sensitivity to standard chemotherapies. Given that glycogen metabolism is linked to chemo-resistance (e.g., in ovarian clear-cell carcinoma and other malignancies), targeting this pathway could enhance treatment efficacy. Our cell line provides a controlled system to test that hypothesis in vitro.

In summary, we introduced a GYS1 gene into ovarian cancer cells using the ABBIE Cas9-guided integrase technology and obtained a stable overexpressing clone. These methodological advances bypass the need for viral transduction and avoids creating DNA double-strand breaks in the host genome. The integration site analysis indicates that while the editing was not perfectly specific for the intended locus using a CRISPR/Cas9-based gRNA design, the resulting SKOV3-GYS1 cell line is a powerful new tool for cancer metabolism research and drug discovery. It exemplifies how integrase-based genome engineering can rapidly generate stable cell models for challenging targets like metabolic enzymes, accelerating the evaluation of novel therapeutic strategies such as GYS1 inhibition. As SOHM and others develop more refined ABBIE reagents and guide RNA designs, we anticipate broader adoption of this platform for creating custom cell lines with user-defined genetic modifications, thereby advancing both basic research and translational applications in oncology.

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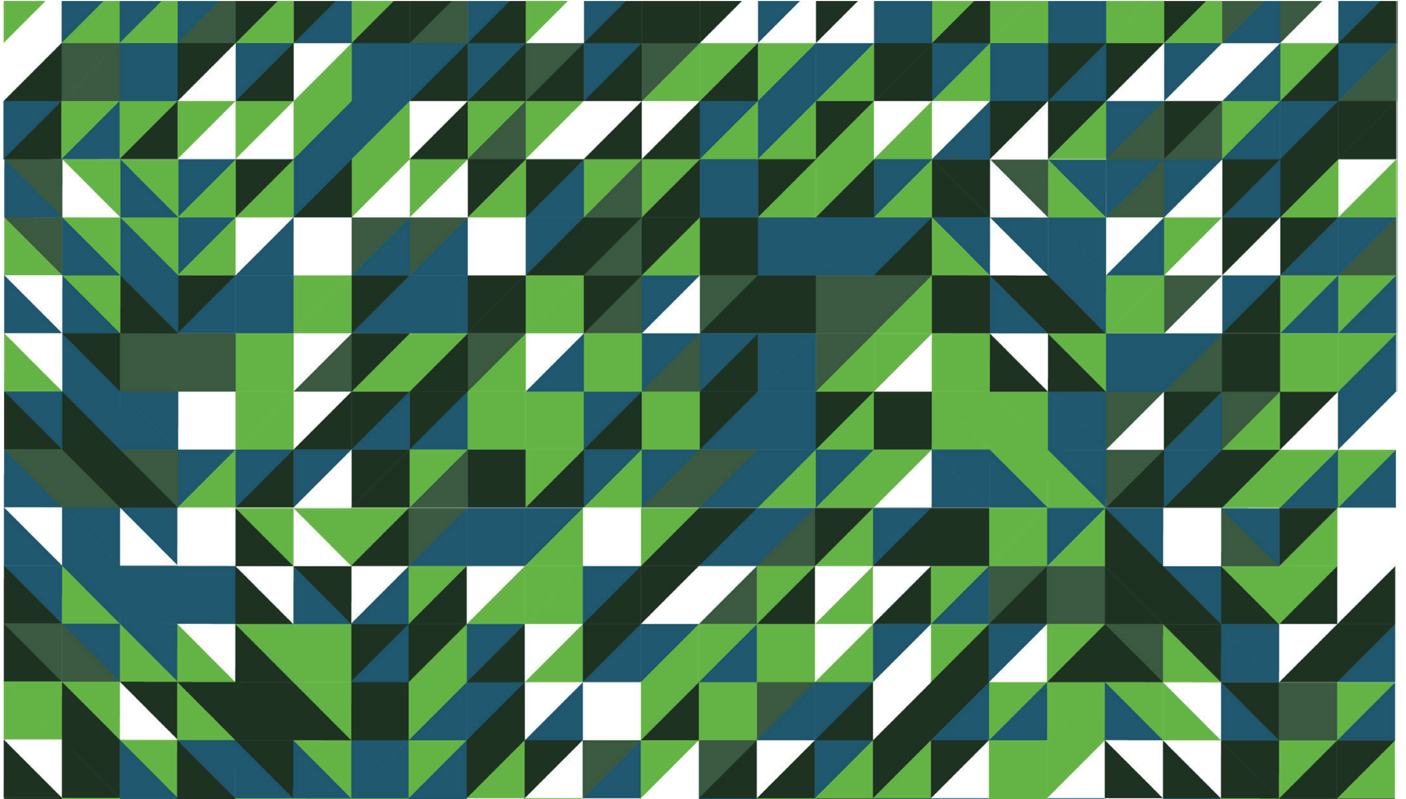

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