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Predicting HDR Competency in Human Embryos via Transcriptomic Profiling of DNA Repair Pathways

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ABSTRACT

Genome editing in early human embryos holds transformative potential but remains limited by the cell's natural tendency to repair DNA breaks using error-prone pathways like non-homologous end joining (NHEJ). In contrast, homology-directed repair (HDR)—required for precise genome editing—is less efficient and poorly characterized in embryonic contexts. In this study, a computational analysis of publicly available single-cell RNA-sequencing data (GSE66507) from 30 human blastocyst cells to profile the transcriptional activity of DNA repair genes is performed. The key players in NHEJ (e.g., *XRCC6, XRCC4*) and HDR (e.g., *RAD51, BRCA1*) across three embryonic lineages: trophectoderm (TE), epiblast (EPI), and primitive endoderm (PE) are examined. The results show that NHEJ-related genes are consistently expressed across all cells, suggesting a default repair mechanism during preimplantation development. HDR genes, on the other hand, show greater variability and are selectively enriched in EPI cells—pointing to a potential transcriptional window of increased HDR activity following embryonic genome activation (EGA). Principal component analysis (PCA) further confirms lineage-specific clustering driven by repair gene expression. These findings highlight how transcriptional profiling can offer valuable clues about repair pathway preferences in early embryos—and guide genome editing strategies toward higher precision and efficiency.

Keywords: *Homology-directed repair (HDR), non-homologous end joining (NHEJ), genome editing, CRISPR-Cas9, single-cell RNA sequencing (scRNA-seq), human blastocyst, embryonic genome activation (EGA), DNA repair pathways, transcriptomic profiling, epiblast (EPI)*

Key Highlights

- NHEJ genes are consistently expressed across all embryonic lineages in the blastocyst.
- HDR gene expression is more variable and enriched in epiblast (EPI) cells.
- PCA reveals lineage-specific clustering based on DNA repair gene expression.
- These transcriptional patterns suggest a developmental window for HDR-based editing.
- Findings support timing- and lineage-targeted CRISPR strategies in early embryos.

1. INTRODUCTION

CRISPR-Cas9 genome editing has revolutionized developmental biology and reproductive medicine by allowing researchers to make precise genetic modifications. However, the success of such editing hinges on how the cell repairs DNA breaks: either through quick but error-prone non-homologous end joining (NHEJ), or through precise, template-based homology-directed repair (HDR). In early embryos, NHEJ typically dominates—limiting the efficiency of accurate gene correction.

Although researchers have explored strategies to boost HDR—such as using small molecules like RS-1 or timing the edit with the cell cycle—applying these methods to human embryos remains challenging due to ethical and technical barriers. One key insight is that the cell's choice of repair pathway may be influenced by developmental timing and cell lineage.

Embryonic Genome Activation (EGA), when the embryo begins widespread transcription, marks a shift in cellular dynamics. The emergence of transcriptionally active and lineage-specified cells—such as trophectoderm (TE), epiblast (EPI), and primitive endoderm (PE)—may also change how these cells respond to DNA damage.

With the availability of single-cell RNA sequencing (scRNA-seq) data, we now have a non-invasive tool to profile the expression of DNA repair genes in individual cells. This opens the door to computationally predicting when and where HDR might be favored, without disrupting embryonic development. In this study, I aim to map these transcriptional signatures and

explore their implications for precise genome editing.

Related Work and Motivation

Understanding when and where DNA repair pathways are active in human embryos is central to improving genome editing outcomes. A number of foundational studies have shaped our knowledge of early human development using single-cell RNA sequencing (scRNA-seq). Petropoulos et al. (2016) characterized transcriptional diversity in preimplantation embryos and defined lineage-specific gene signatures in trophectoderm (TE), epiblast (EPI), and primitive endoderm (PE) cells. Similarly, Yan et al. (2013) provided a developmental timeline of human embryos from zygote to blastocyst, illuminating the dynamics of embryonic genome activation (EGA). Blakeley et al. (2015) extended this work by integrating single-cell gene expression with lineage commitment markers to resolve the early specification of embryonic lineages.

While these studies were instrumental in profiling embryonic transcriptional landscapes, they did not specifically examine DNA repair pathways or their potential role in genome editing. Other studies have begun to explore this connection. Kim et al. (2017) demonstrated that genome editing outcomes in human embryos are strongly influenced by cell type and developmental timing, suggesting that repair competency is not uniform. In parallel, Wells et al. (2019) advocated for the use of gene editing over embryo selection in preimplantation genetic diagnosis (PGD), emphasizing the clinical importance of achieving precise edits—something that hinges on effective HDR.

Recent insights into chromatin dynamics and repair regulation have added another layer of complexity. Zhang et al. (2019) showed that chromatin accessibility is a major determinant of HDR efficiency, and that manipulating these states can improve editing precision. Similarly, Gao et al. (2018) and DeWitt et al. (2016) highlighted how small molecules and protein-level interventions can shift repair pathway preference toward HDR. Meanwhile, studies in oocyte aging, such as by Szymanska et al. (2024), show that repair capacity can be affected by developmental stage and cellular stress—again pointing to the importance of biological context.

Despite this growing body of knowledge, there is still a lack of systematic analysis connecting repair gene expression to specific embryonic lineages or developmental windows. This study addresses that gap by using a computational approach to profile DNA repair gene activity across individual blastocyst cells. My aim is to offer actionable insight into the timing and targeting of CRISPR genome editing—especially in settings where experimental manipulation is limited.

Methods

- **Dataset**: GSE66507 (Petropoulos et al., 2016), containing 30 single-cell RNA-seq profiles from human blastocyst cells.
- **Gene Selection**: 7 NHEJ genes (e.g., *XRCC6*, *LIG4*) and 7 HDR genes (e.g., *RAD51*, *BRCA1*).
- **Analysis**: Log-normalized expression, heatmap visualization, PCA clustering, and expression trends by lineage.

2. RESULTS

3.1 NHEJ Genes Are Highly Expressed Across Lineages

Analysis of scRNA-seq data revealed consistent expression of NHEJ-related genes across TE, EPI, and PE lineages. This supports the idea that NHEJ is transcriptionally active across early embryonic cells, functioning as the default DNA repair system.

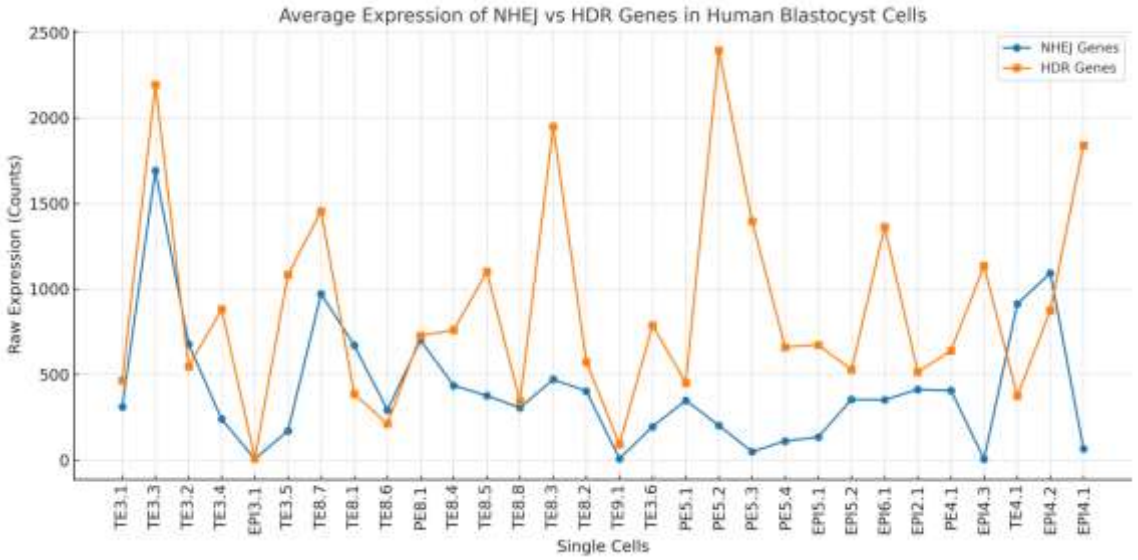

Figure 1 - Average Expression of NHEJ and HDR Genes Across Human Blastocyst CellsLine plot comparing average raw expression (read counts) of non-homologous end joining (NHEJ) and homology-directed repair (HDR) genes across 30 single cells from human preimplantation embryos. NHEJ genes show consistently higher expression across all lineages, while HDR genes exhibit variable expression patterns, with some increase in epiblast (EPI) cells.

3.2 HDR Gene Expression Is Lineage-Specific and Variable

In contrast, HDR genes displayed heterogeneous expression. While some—like *RAD51* and *BRCA1*—were expressed in subsets of cells, they were primarily enriched in EPI cells, suggesting lineage-specific HDR competency.

Figure 2 -Heatmap of DNA Repair Gene Expression in Human Blastocyst CellsLog-transformed expression levels of 14 selected DNA repair genes (7 NHEJ and 7 HDR) across single cells representing trophectoderm (TE), epiblast (EPI), and primitive endoderm (PE) lineages. The heatmap highlights consistently expressed NHEJ machinery and lineage-specific variability in HDR-related transcripts.

3.3 PCA Reveals Lineage-Specific Clustering

Principal component analysis of all 14 genes showed clear lineage-based clustering, particularly distinguishing EPI cells by their elevated HDR expression patterns.

Figure 3 - Principal Component Analysis (PCA) of DNA Repair Gene ProfilesPCA plot showing dimensionality reduction of gene expression profiles based on DNA repair genes. Cells cluster by lineage, with EPI cells showing greater separation potentially due to increased HDR-related transcriptional activity. This spatial distribution reflects functional divergence in DNA repair machinery during early embryonic lineage commitment.

3. DISCUSSION

4.1 NHEJ as the Default Repair Pathway in Early Embryos

The results reinforce what many experimental studies have suggested: non-homologous end joining (NHEJ) is the primary repair mechanism during the earliest stages of human development. High and consistent expression of NHEJ-related genes across all blastocyst lineages suggests that this pathway serves as a baseline mechanism, particularly in cells with limited access to homologous templates.

4.2 HDR Is Lineage-Dependent and Possibly Regulated by EGA

Unlike NHEJ, HDR gene expression varied widely. Key HDR genes such as *RAD51* and *BRCA1* were more active in epiblast (EPI) cells—lineages associated with pluripotency and transcriptional richness. This suggests a potential post-EGA window where cells might be more amenable to precise editing. Chromatin state and transcriptional activity have both been implicated in HDR efficiency (Zhang et al., 2019), which supports this hypothesis.

4.3 Implications for CRISPR Genome Editing in Embryos

This study provides a data-driven roadmap for improving CRISPR editing in early embryos. By identifying HDR-enriched states, researchers could better time or target their interventions. In addition, several studies have demonstrated that molecules like RS-1 or DNA-PK inhibitors can tip the balance toward HDR (Gao et al., 2018; DeWitt et al., 2016). If combined with transcriptomic screening, such strategies could minimize off-target edits and reduce mosaicism.

4.4 Limitations and Future Work

As a transcriptomic analysis, this work is limited to mRNA expression levels. It does not capture protein abundance, post-translational modifications, or actual DNA repair outcomes. Future work could integrate proteomics, chromatin accessibility (e.g., ATAC-seq), or live-cell imaging to validate these predictions. Still, this computational approach offers a low-cost, ethically acceptable strategy to explore repair readiness in human embryos—an area where direct experimentation is often restricted.

4. CONCLUSION

This study used publicly available single-cell transcriptomic data to explore DNA repair pathway expression in early human embryos. The findings suggest that NHEJ is the default repair pathway across all lineages, while HDR activity is more restricted—likely emerging post-EGA in pluripotent EPI cells.

These insights offer a shift in perspective: rather than asking *if* HDR is possible in embryos, we should be asking *when* and *where* it is biologically favored. By mapping these transcriptional cues, researchers can begin designing more precise, context-aware genome editing strategies. This computational framework may also support virtual screening of HDR-enhancing interventions—offering a non-invasive route to improve editing outcomes in early development.

REFERENCES

[1] Petropoulos, S., et al. (2016). Single-cell RNA-seq reveals lineage and transcriptional diversity in human preimplantation embryos.Cell, 165(4), 1012–1026. https://doi.org/10.1016/j.cell.2016.03.014

[2] Yan, L., et al. (2013). Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells.Nature Biotechnology, 31(8), 652–658. https://doi.org/10.1038/nbt.2660

[3] Blakeley, P., et al. (2015). Defining the three cell lineages of the human blastocyst by single-cell RNA-seq.Development, 142(18), 3151–3165. https://doi.org/10.1242/dev.123547

[4] Kim, D., et al. (2017). Genome editing reveals a role for OCT4 in human embryogenesis.Nature, 550, 67–73. https://doi.org/10.1038/nature24033

[5] Wells, D., Vermeesch, J. R., & Simpson, J. L. (2019). Gene editing should replace embryo selection following PGD.Prenatal Diagnosis, 39(7), 545–552. https://doi.org/10.1002/pd.5469

[6] Szymanska, K. J., et al. (2024). The role of declining ATM function in oocyte aging.Cell Death & Disease, 15, 171. https://doi.org/10.1038/s41420-024-02041-z

[7] Zhang, Y., et al. (2019). Enhancing CRISPR/Cas9 genome editing by manipulating chromatin accessibility.Cell Reports, 26(7), 1875–1887.e5. https://doi.org/10.1016/j.celrep.2019.01.054

[8] Gao, Y., et al. (2018). Enhancing homology-directed genome editing by modulating DNA repair pathway choice.Nature Methods, 15, 467–474. https://doi.org/10.1038/s41592-018-0092-0

[9] DeWitt, M. A., et al. (2016). Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells.Nature Biotechnology, 34(3), 320–328. https://doi.org/10.1038/nbt.3462

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