Gene Set Enrichment Analysis of an Osteosarcoma Dataset Reveals Potential Immune-Related Therapeutic Targets in Osteosarcoma

Bragadheshwaran KK

Abstract
Osteosarcoma (OS) is a rare but aggressive bone cancer with poor survival rates in metastatic cases. To explore potential therapeutic targets, we analyzed the GSE1153 gene expression dataset of U2OS osteosarcoma cells expressing estrogen receptor alpha (ERα) or beta (ERβ) treated with estradiol. Principal component analysis (PCA) was used to identify global expression patterns, and gene set enrichment analysis (GSEA) was applied to uncover biological pathways enriched in these data. The most enriched pathways were related to DNA repair and protein modification, including SUMOylation, homologous recombination, base excision repair, and resolution of abasic sites. These findings suggest that osteosarcoma cells may rely on enhanced DNA repair and stress-response mechanisms for survival, which could contribute to treatment resistance and represent potential therapeutic targets.

1. Introduction

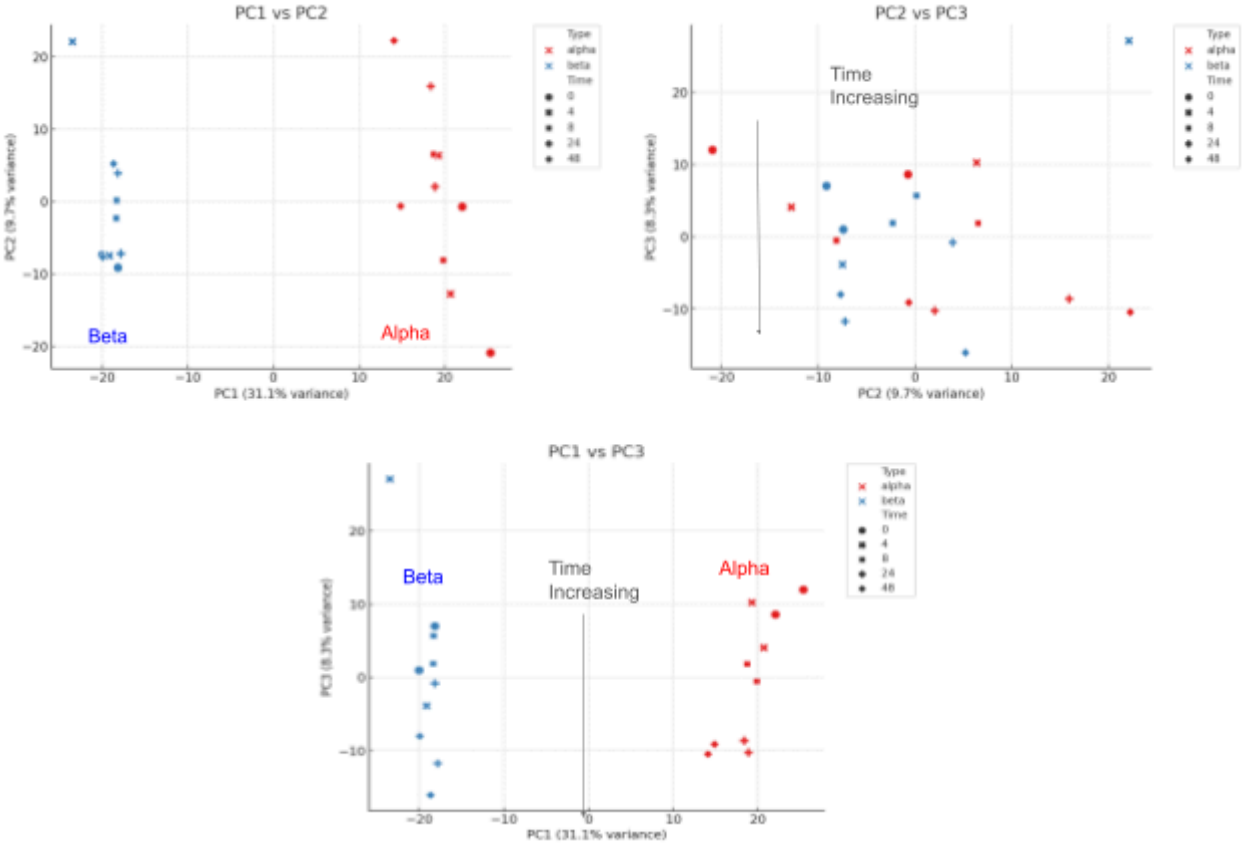
Osteosarcoma (OS) is the most common primary bone cancer in children and adolescents. Although rare, it is highly aggressive, with survival rates dropping from around 70 % in localized cases to below 25 % in patients with metastasis [1]. Current treatments rely on surgery and chemotherapy, but outcomes for advanced or relapsed cases have not significantly improved in decades. This highlights the urgent need for new therapeutic approaches. One potential strategy is to better understand how osteosarcoma interacts with the immune system at the molecular level. Gene expression datasets provide opportunities to explore these mechanisms, revealing pathways that may serve as targets for immunotherapy or drug development [1]. In this study, we analyzed a publicly available dataset of osteosarcoma cells expressing different estrogen receptor subtypes. Using PCA and GSEA, we aimed to identify biological pathways—particularly immune-related and DNA-repair pathways—that may represent vulnerabilities in osteosarcoma.

2. Methods

This study used the publicly available GSE1153 dataset from the NCBI Gene Expression Omnibus (GEO) [1]. The dataset contains gene expression profiles from U2OS human osteosarcoma cells engineered to express either estrogen receptor alpha (ERα) or estrogen receptor beta (ERβ). Cells were treated with 17β-estradiol (E2) and collected at five time points: 0, 4, 8, 24, and 48 hours. Gene expression was measured using the Affymetrix Human Genome U95A GeneChip platform. Metadata on ER subtype and collection time was used for grouping samples. All analyses were performed in Python using pandas, scikit-learn, matplotlib, and seaborn. The expression matrix was transposed so that samples were rows and genes were columns, then standardized with StandardScaler. PCA was performed with three components, and scatter plots of PC1 vs PC2, PC2 vs PC3, and PC1 vs PC3 were generated (Figure 1). PCA loadings for PC1–PC3 were exported as .rnk files for enrichment analysis. GSEA was

conducted using easyGSEA within the eVITTA platform [3]. Ranked PCA loadings were tested against multiple pathway databases, with a focus on Reactome (RA) due to its coverage of immune and DNA-repair processes [2]. Pathways with $p < 0.1$ were considered significant. Results were visualized using bar plots and enrichment networks (Figures 2–3).

3. Results



The most enriched pathways identified from the Reactome database were RA_SUMO_E3_ligases_SUMOylate_target_proteins, RA_HDR_through_Homologous_Recombination, RA_Base_Excision_Repair, and RA_Resolution_of_Abasic_Sites (Figure 2). All four pathways showed strong positive enrichment, indicating upregulation in the dataset [2]. PCA demonstrated a clear separation between sample groups, particularly between ERα and ERβ conditions across treatment times (Figure 1). This separation supports that the enriched pathways reflect distinct underlying gene-expression programs triggered by estrogen receptor activation [1]. The GSEA bar plot confirmed these trends, showing positive enrichment scores for DNA-repair and SUMOylation pathways (Figure 3). The consistent clustering of these pathways suggests strong functional overlap and coordinated regulation in osteosarcoma cells [3].

4. Discussion

Our analysis highlights DNA-repair and protein-modification pathways as key features of gene regulation in osteosarcoma cells. The upregulation of homologous recombination and base-excision repair suggests that osteosarcoma cells invest heavily in maintaining genome

stability [1]. This may allow them to survive DNA damage from chemotherapy, potentially contributing to treatment resistance. Similarly, SUMOylation is known to regulate stress responses and transcriptional programs, and its enrichment here suggests it may play a role in tumor survival [2]. While this study focused primarily on Reactome pathways, immune-signaling pathways such as IL-6, IL-17, and Toll-like receptor cascades also appeared in earlier analyses. These findings suggest that osteosarcoma progression involves both immune signaling and DNA-repair networks, which may provide opportunities for combined therapeutic targeting [3]. Limitations of this study include the reliance on a single dataset (GSE1153) and the lack of experimental validation. Future studies should incorporate additional datasets, including single-cell RNA sequencing, and test these pathways in laboratory or clinical settings.

5. Conclusion

This study demonstrates how gene-expression analysis and pathway enrichment can reveal biological processes relevant to osteosarcoma. The upregulation of SUMOylation and DNA-repair pathways suggests mechanisms by which osteosarcoma cells maintain survival and resist therapy. These results point to DNA-repair and immune-related pathways as potential therapeutic targets, supporting the continued development of precision approaches in osteosarcoma treatment.

6. References

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