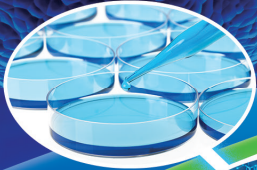


Whitepaper:

Application of Synthetic Peptide Growth Factors for iPSC-based Hematopoietic Stem Progenitor Cells (HSPCs) and NKT Cells



PeptiGrowth's Synthetic Peptide Growth Factors—*Forging the Path Forward for Cell Therapy and Regenerative Medicine*

Induced Pluripotent Stem Cell (iPSC) technology has revolutionized the field of regenerative medicine, offering unprecedented opportunities for developing novel cell-based therapies. Among these, the generation of various hematopoietic lineage cells from iPSCs via hematopoietic stem/progenitor cells (HSPCs) represents a promising approach. Therapies are being designed for creating advanced cell therapy solutions for cancer immunotherapy by enabling expansion of functional T-lymphocytes, such as cytotoxic T lymphocytes, natural killer T (NKT) cells, macrophages and microglia in a mass production setting.

By generating HSPCs from iPSCs, we can develop a variety of gene-edited cell therapy products, such as CAR-T cells, CAR-NK cells, and NKT cells. However, the current use of autologous iPSCs presents several challenges: the generation process is time-consuming, costly, and prone to variability due to patient-specific factors, making it difficult to standardize and scale.

In contrast, allogeneic iPSC-derived cellular products offer significant advantages. They can be produced in large batches from a single donor, providing scalability, cost-effectiveness, and rapid availability, with the added benefit of standardized quality and reduced variability.

Nonetheless, the production of allogeneic HSPCs from iPSCs and their differentiation into specific T lymphocytes as well as their expansion still require use of many different types of growth factors and cytokines. These factors are essential for ensuring the stability and consistency of the cell products, but their high cost contributes substantially to overall production expenses, which could limit the widespread adoption of iPSC based cell therapies.

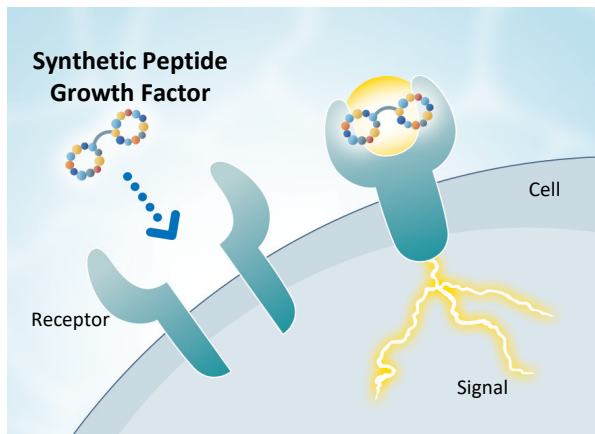


Fig. 1 Schematic illustration of the working mechanism of Synthetic Peptide Growth Factors, PG-peptides.

Synthetic Peptide Growth Factors, or PG-peptides, developed by PeptiGrowth Inc., exhibit activity comparable to recombinant growth factors and cytokines (**Fig.1**). PG-peptides consist of denovo identified highly stable cyclic peptides, which provide excellent stability during culturing and storage. These entirely chemically synthesized peptides offer superior consistency in product quality and activity compared to recombinant proteins. They are free from animal-derived components which makes them particularly suitable for use in cell therapy products. Additionally, their scalable production results in lower costs, allowing them to be offered at cost effective pricing. Consequently, PG-peptides have significant potential to advance iPSC-based cell therapies for cancer treatment.

In this paper, we highlight PG-peptides—**PG-007, PG-008, and PG-010**—as synthetic peptide growth factors for VEGF, Wnt3a, and TPO, respectively, and explore their potential role in advancing iPSC-based cell therapy.

Wnt3a alternative peptide, PG-008, is composed of two distinct cyclic peptides: one binds to the Frizzled receptor, and the other to the LRP5/6 receptor, together exhibiting agonistic activity on the β -catenin pathway of Wnt signaling. Recombinant Wnt3a is notoriously difficult to handle and shows significant lot-to-lot variability, primarily due to its lipid moieties, which cause it to aggregate and lose activity easily. PG-008 overcomes these challenges by achieving potent agonistic activity on the β -catenin pathway through its peptide based structure, making it more stable and effective than recombinant Wnt3a.

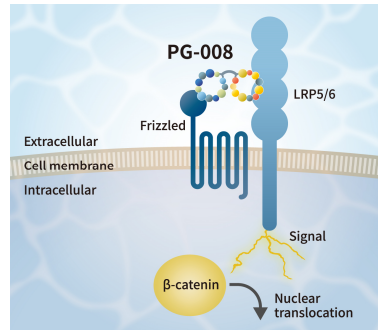


Fig. 2 Schematic illustration of the working mechanism of PG-008.

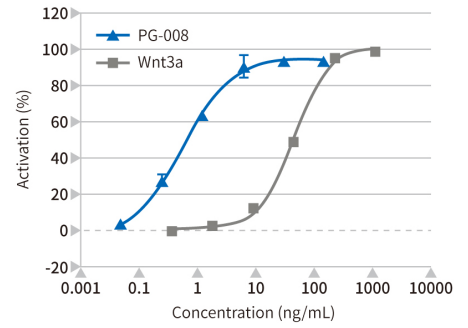


Fig. 3 TCF-LEF reporter assay using HEK293 cells. PG-008 activated the reporter gene at a lower concentration than Wnt3a, demonstrating that PG-008 has superior agonistic activity (over 50 times greater) compared to Wnt3a.

No Cytotoxicity: CHIR99021, a small molecule commonly used as a Wnt3a agonist, activates the β -catenin pathway in Wnt signaling by inhibiting GSK3 β , but it often causes cytotoxicity, leading to low cell yields. In contrast, PG-008 has been proven to exhibit no cytotoxicity, even at the highest tested concentration of 10 μ M (Fig.4). As a result, PG-008 is a superior Wnt3a agonist, offering the potential for higher cell yields compared to CHIR99021.

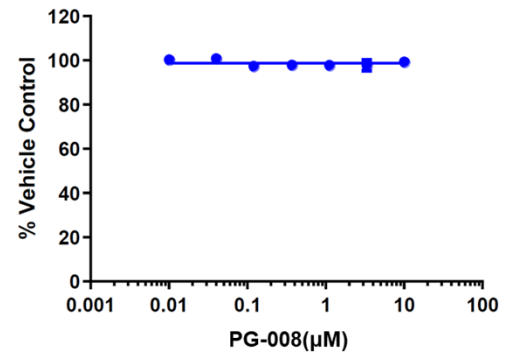


Fig. 4 Cytotoxicity evaluation of PG-008 using HEK293 cells. No significant cytotoxicity was observed even at concentrations up to 10 μ M of PG-008.

VEGF alternative peptide, PG-007, is a homodimeric cyclic peptide exhibiting specific binding ability and agonistic activity against human VEGFR2 (vascular endothelial growth factor receptor 2). VEGF is well-known for its involvement in angiogenesis and the formation of blood vessels, but its impact extends to hematopoiesis, where it influences the differentiation and maturation of HSPCs. PG-007 can be used in the same application as recombinant VEGF with its specific agonistic activity of human VEGFR2.

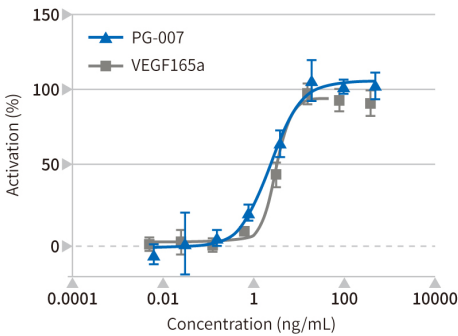
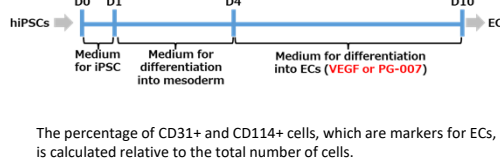


Fig. 5 Proliferation assay of iPSC-derived endothelial cells. PG-007 showed activity comparable to recombinant VEGF165a in promoting cell proliferation.

Differentiation of iPSCs into endothelial cells (ECs)

Protocol overview



The percentage of CD31+ and CD114+ cells, which are markers for ECs, is calculated relative to the total number of cells.

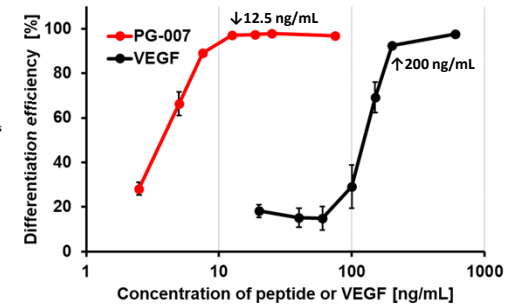


Fig. 6 Differentiation efficiency of human iPSCs into endothelial cells (ECs) was evaluated when using recombinant VEGF or PG-007. PG-007 achieved the same level of differentiation efficiency as VEGF at 1/16th of the concentration.

TPO alternative peptide, PG-010, is a homodimer of a cyclic peptide that binds to the human TPOR (thrombopoietin receptor), functioning as a TPOR agonist. Notably, PG-010 achieves effectiveness comparable to that of TPO at 2.6 times lower concentration.

The applications of PG-010 are diverse. It effectively promotes the proliferation and maturation of megakaryocytes, which are crucial for platelet production. Additionally, PG-010 facilitates the differentiation of hematopoietic stem progenitor cells (HSPCs) from pluripotent stem cells, as well as the proliferation of HSPCs, thereby supporting their expansion and enhancing their functionality.

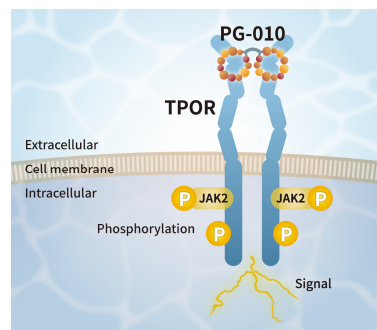


Fig. 7 Schematic illustration of the working mechanism of PG-010. Upon binding to TPOR, PG-010 activates the JAK2-STAT5 signaling pathway.

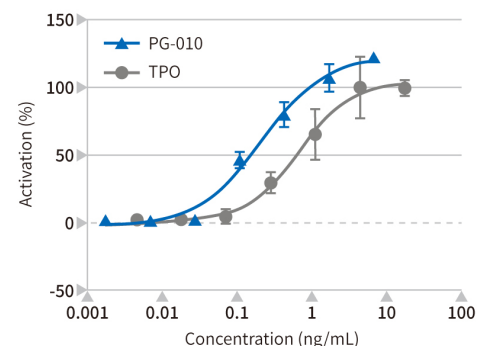


Fig. 8 Reporter gene activation assay on the JAK2-STAT5 signaling pathway using TPO or PG-010. PG-010 exhibits a comparable level of gene activation at 2.6 times lower concentration than recombinant TPO.

Doubled Yields of NKT Cells with Equivalent Differentiation Efficiency

After the initial differentiation stage, HSPCs were harvested and reseeded at the same density for both the control and PG-peptide conditions. The subsequent differentiation into NKT cells was performed under identical conditions for both samples. Notably, NKT cells derived from HSPCs differentiated with PG-peptides yielded **more than double the number of cells** during the second differentiation stage, while maintaining equivalent differentiation efficiency (Fig. 11).

Results of the 2nd differentiation stage (HSPCs→NKT)

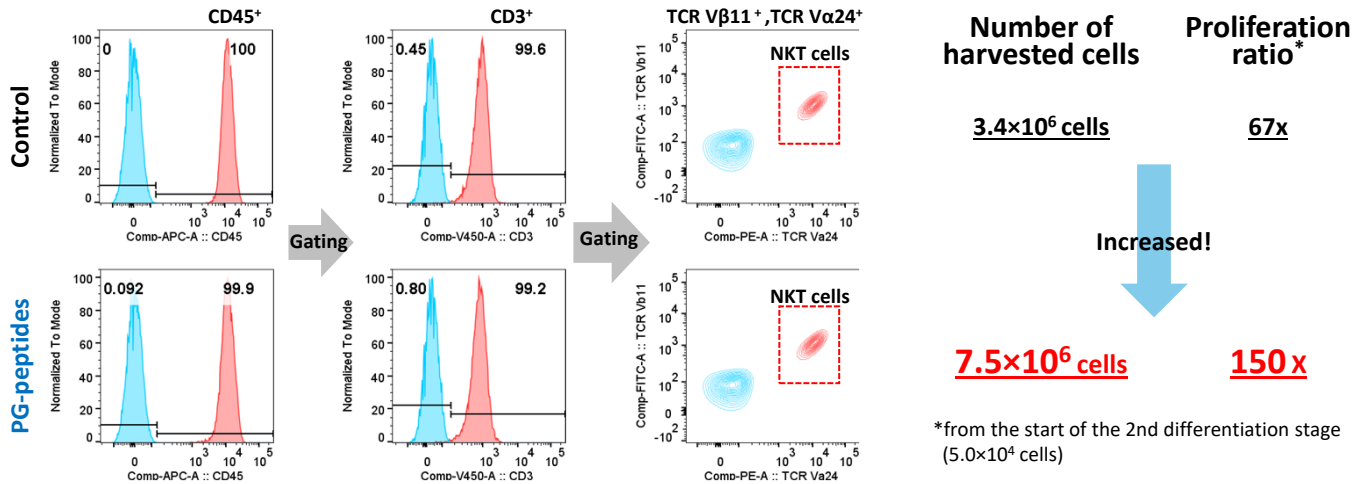


Fig. 11 Measurement of NKT cell surface markers and cell counting results for harvested NKT cells.

Equivalent Cell Functionality

The functionality of the NKT cells was assessed by evaluating their cell killing activity against cancer cells and measuring IFN γ secretion levels. NKT cells derived from HSPCs using PG-peptides exhibited cell killing activity and IFN γ secretion comparable to those produced under control conditions, indicating that **the functionality of the cells remained equivalent** (Fig. 12).

In summary, the use of PG-007, PG-008, and PG-010 significantly enhanced the efficiency of NKT cell differentiation from iPSCs, reducing the time required and increasing the number of cells obtained. This demonstrates the potential of PG-peptides to improve cell therapy processes without compromising cell functionality.

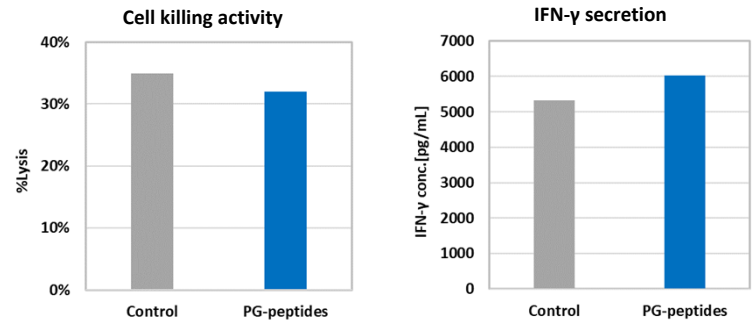


Fig. 12 Functionality assessment of NKT cells. In vitro cell killing assay against cancer cells and measurement of IFN γ secretion levels from NKT cells were conducted to evaluate NKT cell functionality.

“Remarkable improvement of NKT cell production with PG-peptides has been truly impressive. We would conduct further experiments for their introduction in our manufacturing processes.” Dr. Koseki

As demonstrated, the use of PG-peptides has the potential to significantly contribute not only to iPSC-based NKT cells but also to other cell therapies such as CAR-T and CAR-NK cells. If you are interested in PG-007, PG-008, and PG-010 introduced in this paper, or any other PG products, please contact us through the details provided below.

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