Targeted Thyroid Cancer Therapy Using Peptide-Loaded Hydrogels

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Abstract

Thyroid cancer is the most prevalent endocrine malignancy globally, with papillary thyroid carcinoma being the dominant subtype and exhibiting a rising incidence. Mutations in the BRAF gene are responsible for the majority of thyroid cancers. The BRAF gene encodes the BRAF protein, which plays a role in the MAPK/ERK signaling pathway that regulates cell growth and division. The BRAF mutation causes continuous activation of the BRAF protein, resulting in constant MEK and ERK activation. This leads to uncontrolled cell division and tumor formation. Conventional treatments such as thyroidectomy and radioiodine ablation lack tumor specificity and often harm healthy tissues. Therefore, there is a need for therapeutic strategies that can slow down tumor development and selectively kill cancer cells without damaging healthy tissues. DR8 peptide is an antifibrotic agent that functions by inhibiting the MAPK/ERK pathway involved in fibrosis formation. Considering the mechanism of tumor formation in papillary thyroid carcinoma, it is hypothesized that the DR8 peptide may also inhibit cancerous cell growth and induce cell death. In this study, a functional self-assembling peptide (SAP) hydrogel including DR8 peptide is designed for the treatment of papillary thyroid carcinoma. The effectiveness of the developed hydrogel system was evaluated on BCPAP and L929 cell lines. Cells were incubated with the soluble form of the DR8 peptide at various concentrations. While the DR8 peptide induced 40–50% cancer cell death with 40 μM DR8, it exhibited no cytotoxicity on healthy cells. Since the effectiveness of the DR8 peptide was proven on the BCPAP cell line, an injectable SAP hydrogel system consisting of KLDL SAP and the DR8 peptide was developed by synthesizing KLDL and DR8 end-to-end with Gly-Gly-Gly linkers. Future studies will b conductd to explore the effect of KLDL/DR8 hydrogels on BCPAP and L929 cell lines.

Keywords: Papillary thyroid carcinoma, MAPK/ERK signaling pathway, peptides, self-assembling peptide hydrogels

Introduction

Thyroid cancer is an endocrine malignancy that affects women approximately four times more frequently than men ((Al-Brahim, Asa, & medicine, 2006; Iyengar, 2019) . Despite its relatively high prevalence, it generally has a low mortality rate. However, its treatment often involves ionizing radiation, management of goiter, and consideration of reproductive factors (Al-Brahim et al., 2006; Albi et al., 2017). Therefore, advanced treatments are being investigated, currently. Thyroid cancer is primarily initiated by mutations activating the mitogen-activated protein kinase (MAPK) signaling cascade, and the mutations in the BRAF gene are the cause of the majority of thyroid cancers (Mondragon-Teran et al., 2016; Wang et al., 2021). MAPKs plays a significant role in cell proliferation, and ERK, a subgroup of MAPK contributes to the EMT progression by providing a feedback loop. The BRAF gene is responsible of encoding a serine/threonine kinase in the MAPK/ERK pathway, which is crucial in cell proliferation (Mondragon-Teran et al., 2016; Pilat, Bumma, Back, Pandit, & Weise, 2018). When BRAF gene is mutated, especially the V600E substitution (substitution of valine to glutamic acid at codon 600), ERK remains constantly active leading to abnormal activation of the MAPK/ERK pathway (Śmiech, Leszczyński, Kono, Wardell, & Taniguchi, 2020; Wellbrock & Hurlstone, 2010). Continuous activation results in uncontrolled cell division, and inhibits apoptosis. Therefore inhibiting MAPK/ERK pathway is an efficient method for overcoming thyroid cancer.

In recent years, peptide-based therapeutics have gained considerable attention as a new generation of targeted agents in cancer treatment. Peptides offer high biocompatibility, receptor specificity, and ease of synthesis and modification. DR8 peptide (DHNNPQIR) is an antifibrotic peptide effective in reducing fibrosis in pulmonary and renal fibrosis models (Wang et al., 2021; Wang et al., 2019). DR8 functions by suppressing TGF-B/MAPK and relatively ERK, p38 MAPK pathway. Considering the underlying mechanisms of thyroid cancer progression, the DR8 peptide is proposed to inhibit tumor

development and induce cancer cell death. However, peptides administered systemically often face limitations such as enzymatic degradation, rapid clearance, and poor bioavailability. To address these issues, self assembling-peptide (SAP) hydrogels have been developed as injectable, biocompatible drug delivery systems offering localized and sustained release of bioactive agents. These hydrogels mimic the extracellular matrix, enhance peptide stability, and reduce systemic toxicity (Chen & Zou, 2019). In this study, we propose a novel SAP hydrogel composed of KLDL and DR8 peptide synthesized end-to-end. The aim is to target papillary thyroid carcinoma by targeting the tumor site locally by a non-invasive method. The effect of the soluble form of DR8 peptide is investigated on human papillary thyroid carcinoma cells (BCPAP) and mouse fibroblast cells (L929). Rheological analysis was conducted to assess the mechanical stability and viscoelastic behaviour of KLDL-DR SAP hydrogel. Further investigations with KLDL-DR8 hydrogel on BCPAP and L929 cells will be conducted to explore potential clinical applications.

Materials and Methods

1. Solid-Phase Peptide Synthesis

DR8 (DHNNPQIR), KLDL (KLDLKLDLKLDL), and KLDL/DR8 peptides were synthesized using solid-phase peptide synthesis method on Rink Amide NovaGel resin (0.62 mmol/g). For each sequence, 600mg of resin was left to swell in dimethylformamid (DMF) for 30 minutes. Amino acids with Fmoc-protected amine groups (2 eq), 2-(1H-benzotriazole-l-yl)-1,1,3,3-tetramethyluronium Hexafluorophosphate (HBTU; 1.95eq) and *N, N*-diisopropylethylamine (DIEA; 3 eq) were mixed in DMF (9 ml) and added to the resin. The resin was left in contact with the coupling solution for 4-6 hours. After coupling 20% piperidine/DMF solution was used for removing Fmoc protection groups by keeping in contact with the resin for 20 min. After each coupling and deprotection step Kaiser test was performed to detect the presence of amines. If the test results indicated a positive outcome, the resin was rinsed with DMF (5×3 mL), and the coupling reactions were repeated until a negative test result was achieved. Upon obtaining a negative result, the resin was again washed with DMF (5×3 mL) and then treated with 20% piperidine in DMF for 30 minutes to deprotect the Fmoc group. This process was repeated for each subsequent amino acid until the complete peptide sequence was assembled.

Different from KLDL and DR8 sequences, KLDL/DR8 was synthesized end-to-end. To enhance the flexibility of the peptide, after the KLDL sequence is completed, before continuing with DR8, Gly-Gly-Gly sequence is added to the resin. Gly-Gly-Gly sequences are commonly introduced between long peptide fragments to act as flexible linkers. Glycine residues lack side chains, making them highly flexible and non-bulky, which helps reduce steric hindrance between adjacent domains. This improves the overall folding, accessibility, and functional performance of the peptide (Van Rosmalen, Krom, & Merkx, 2017). When all the sequences were complete, cleavage of the peptide from the resin is performed with keeping the resin in contact with cleavage solution (Trifuoroacetic acid (TFA) (95% (v/v)), triisopropylsilane (TIPS) (2.5% (v/v)), deionized water (2.5% (v/v)) for 2 hours. After 2 hours, the solution was poured into cold ether and centrifuged at 4500rpm for 10 minutes, 3 times. The suspension was centrifuged, after which the supernatant was discarded. Any residual ether was eliminated using a vacuum evaporator, and the resulting solid was subsequently freeze-dried through lyophilization.

2. Cytotoxicity Evaluation

To assess cytotoxicity and cell viability of BCPAP and L929 cells in the presence of DR8 peptide in soluble form, MTT assay was performed. L929 and BCPAP cells were cultivated in DMEM including 10% FBS, 100units/ml penicillin and 100 µg/ml streptomycin. DR8 peptide solution were prepared by dissolving DR8 powder in cell culture basal media containing 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were seeded on 96 well plate at a density of 10^4 cells/well and experiments were realized in three repetitions.

Seeded cells were left in contact with 0 µM, 5 µM, 10 µM, 20 µM, 40 µM DR8 for 1 hour and 24 hours for incubation at 37°C. When incubation times were over, solutions were removed, and 100 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours at 37 °C. Then, the formazan crystals formed by metabolically active cells were dissolved by adding 100 µL of DMSO to each well. Absorbance was measured at 570 nm using a microplate reader.

3. SAP Hydrogel Preperation and Characterization

KLDL and KLDL/DR8 SAP hydrogels were prepared in 2% density. Firstly, 20 mg of peptide was dissolved in 500 µL of distilled water. Assembly of the peptides was initiated by adding same amount of Dulbecco's Modified Eagle Medium (DMEM) with 25mM HEPES buffer. The hydrogel were left in incubator at 37 °C for total assembly. Disc-shaped hydrogel samples, prepared using a 20 mm cork borer, were placed onto the Discovery Hybrid Rheometer-2 (HR2, TA Instruments, New Castle, DE) for analysis. The storage modulus (G′) and loss modulus (G″) were recorded using a 0.5 cm gap, 1% strain, and an angular frequency range of 0.1 to 10 rad/s.

4. Statistical Analysis

Statistical analysis was conducted using two-way ANOVA (SPSS 12.0, SPSS GmbH, Germany), followed by the Student–Newman–Keuls test for post hoc comparisons. Each experiment was performed in triplicate, with a minimum of three samples per experimental group (n = 3). Mean values and standard deviations were calculated for all data. Statistical significance between groups was determined based on p-values, with thresholds set at *p < 0.05, **p < 0.01, and *p < 0.001."

Results and Discussion

1. Self-assembled hydrogel characterization

Injectable hydrogels have gained significant attention for regulating cell behaviour in vivo, due to their adjustable functional and mechanical properties (Shirbhate & Bajaj, 2022). KLDL-based hydrogels can effectively load and control the release of drugs. In this regard, in this project KLDL/DR8 SAP hydrogel was developed to provide a novel injectable hydrogel for thyroid cancer treatment. KLDL and KLDL/DR8 SAP hydrogels were produced by using lyophilized KLDL and KLDL/DR8 peptides. Rheology analysis was performed on the hydrogels to analyze the storage modulus (G') and loss modulus (G'') to assess the hydrogel's macroscopic properties. Both hydrogels showed maximum storage values below 2000 Pa. Storage modulus and loss modulus of KLDL/DR8 were observed between 717-1703 and 110-117 Pa, respectively, and these values were 369-451 and 109-115 Pa for KLDL hydrogel. Rheological characterization of both hydrogel formulations showed a storage modulus exceeding loss modulus, indicating a dominant elastic, solid-like behavior (Pulat, Gökmen, Özcan, & Karaman, 2025). This suggests both hydrogels exhibited well-formed, crosslinked networks capable of retaining their structural integrity. The predominance of G' over G" is a hallmark of viscoelastic solids and is desirable in many biomedical applications where mechanical stability and shape retention are critical (Stojkov, Niyazov, Picchioni, & Bose, 2021). This indicates that the designed hydrogels were appropriate for the use of injectable applications.

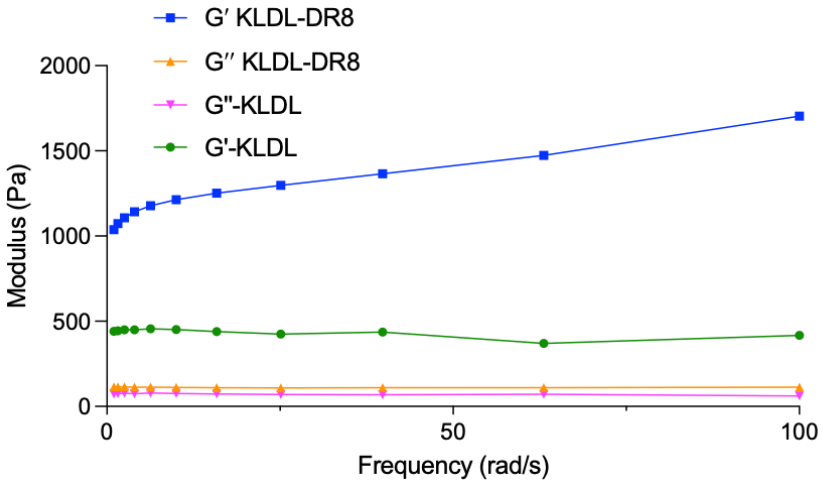

Figure 1. Rheological assessment of the self-assembled peptide hydrogels KLDL, KLDL/DR8, with G′ and G″ indicating the storage and loss modulus, respectively.

2. Cytotoxicity and Cancer Inhibition Evaluation

For the evaluation of the cytotoxicity of DR8 peptide on L929 and BCPAP cells, the soluble form of DR8 was used. Cells were incubated with DR8 solution for 1 and 24 hours. At the end of incubation times, MTT cell viability analysis was performed. L929 cells incubated for 1 hour with DR8 showed no

cytotoxicity, and the cell viability was increased at a ratio of 1% at 5µM, 5% at 10 µM, 3% at 20 µM, and 7% at 40 µM. While cell viability has not changed significantly for L929, a decrease in BCPAP cell viability was observed with the 10 µM DR8. BCPAP cells remained at the same viability as the control. 5 µM, 10 µM, 20 µM, and 40 µM DR8 resulted 5%, 13%, and 19% decreases in cell viability. Compared to 1 h, 24 h incubation exhibited similar results with L929. However, a significant change in BCPAP cells was observed with 24 h incubation. 5 µM, 10 µM, 20 µM, and 40 µM DR8 resulted in 23%, 35%, 40%, and 43% cell death, respectively. The most efficient concentration was determined as 40 µM with a minimum decrease in the viability of healthy cells, and a 43% decrease in the viability of thyroid cancer cells. Other studies in the literature have demonstrated that MEK inhibitors suppressed cell proliferation in BRAF-mutated cells (Schubert, Mariko, Clerc, Huillard, & Groussin, 2023), however did not provide apoptosis (Liu & Xing, 2008). Since the soluble form of DR8 has been shown to induce cell death in BCPAP thyroid cancer cells, the developed hydrogel incorporating DR8 may represent a promising injectable platform for inhibiting thyroid cancer progression.

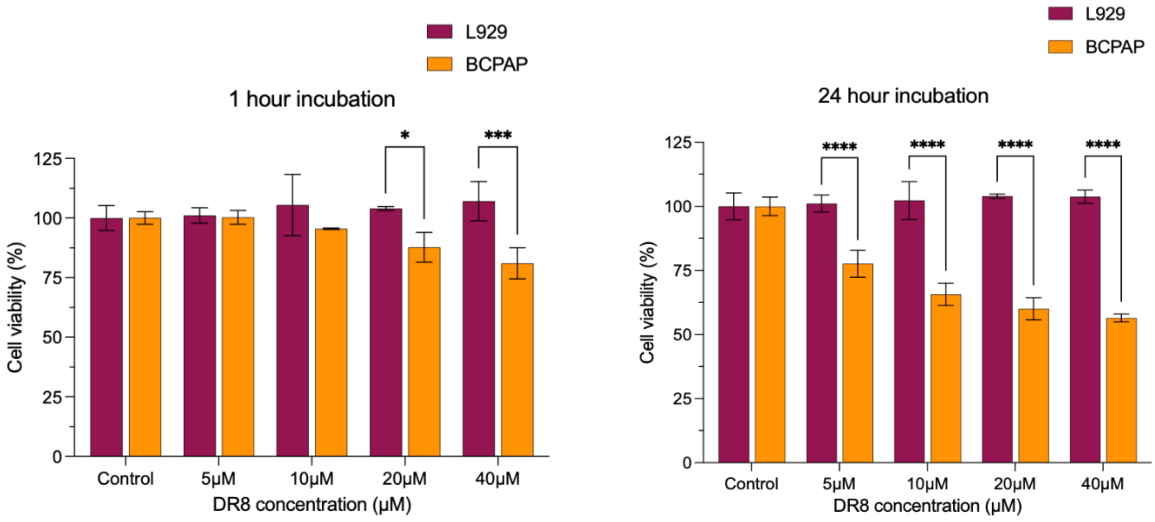

Figure 2. Cell viability percentage of L929 and BCPAP cells incubated with various concentrations of DR8 peptide in basal media for 1 hour and 24 hours (10^4 cells/well). Error bars represent mean ± SD ($n = 5$) Significant differences among groups were evaluated using one-way ANOVA with Newman–Keuls post hoc analysis (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Conclusions

In this study, the biological activity and therapeutic potential of the DR8 peptide were investigated in the context of thyroid cancer, particularly focusing on the inhibition of the TGF-β/MAPK/ERK signaling pathway. The KLDL/DR8 SAP was successfully synthesized using solid-phase peptide synthesis. To ensure structural flexibility and reduce steric hindrance between functional domains, Gly-Gly-Gly linkers were introduced between peptide fragments. Rheological analysis of both hydrogel formulations revealed that the storage modulus was higher than the loss modulus, indicating predominantly elastic, solid-like properties. These results suggest that both hydrogels formed stable structural integrity. BCPAP cells harboring the BRAF V600E mutation, were used to model disease progression in vitro. Given the constitutive activation of the MAPK/ERK pathway driven by this mutation, the DR8 peptide's ability to suppress downstream signaling and promote cancer cell death is of particular importance. Results suggest that DR8 inhibits EMT and cell proliferation, likely by reducing phosphorylation of ERK and interfering with TGF-β receptor mediated activation. In conclusion, DR8 peptide shows strong potential as a therapeutic candidate for thyroid cancer by targeting key oncogenic pathways such as TGF-β/MAPK/ERK. Moreover, its incorporation into hydrogel systems may provide a promising strategy for localized and sustained peptide delivery in future in vivo studies.

References

Al-Brahim, N., & Asa, S. L. (2006). Papillary thyroid carcinoma: an overview. Archives of pathology & laboratory medicine, 130(7), 1057-1062.

Albi, E., Cataldi, S., Lazzarini, A., Codini, M., Beccari, T., Ambesi-Impiombato, F. S., & Curcio, F. J. I. j. o. m. s. (2017). Radiation and thyroid cancer. 18(5), 911.

Chen, J., & Zou, X. J. B. m. (2019). Self-assemble peptide biomaterials and their biomedical applications. 4, 120-131.

Iyengar, S. R. (2019). A Retrospective Study of Clinicopathological Profile and Treatment Outcomes of Thyroid Malignancies Presented to a Tertiary Care Teaching Hospital.

Liu, D., & Xing, M. J. T. (2008). Potent inhibition of thyroid cancer cells by the MEK inhibitor PD0325901 and its potentiation by suppression of the PI3K and NF-κB pathways. 18(8), 853-864.

Mondragon-Teran, P., López-Hernández, L. B., Gutierrez-Salinas, J., Suárez-Cuenca, J. A., Luna-Ceballos, R. I., & Valle-Solís, A. E. J. C. y. C. (2016). Intracellular signalling mechanisms in thyroid cancer. 84(5), 434-443.

Pilat, M., Bumma, N., Back, J., Pandit, T., & Weise, A. J. S. R. (2018). BRAF: From Discovery to Drug Resistance. J. 2, 1007.

Pulat, G., Gökmen, O., Özcan, Ş., & Karaman, O. J. J. o. B. M. R. P. A. (2025). Collagen binding and mimetic peptide-functionalized self-assembled peptide hydrogel enhance chondrogenic differentiation of human mesenchymal stem cells. 113(1), e37786.

Schubert, L., Mariko, M. L., Clerc, J., Huillard, O., & Groussin, L. J. C. (2023). MAPK pathway inhibitors in thyroid cancer: preclinical and clinical data. 15(3), 710.

Shirbhate, U., & Bajaj, P. J. C. (2022). Injectable and self-invigorating hydrogel applications in dentistry and periodontal regeneration: A literature review. 14(9).

Śmiech, M., Leszczyński, P., Kono, H., Wardell, C., & Taniguchi, H. J. G. (2020). Emerging BRAF mutations in cancer progression and their possible effects on transcriptional networks. 11(11), 1342.

Stojkov, G., Niyazov, Z., Picchioni, F., & Bose, R. K. J. G. (2021). Relationship between structure and rheology of hydrogels for various applications. 7(4), 255.

Van Rosmalen, M., Krom, M., & Merkx, M. J. B. (2017). Tuning the flexibility of glycine-serine linkers to allow rational design of multidomain proteins. 56(50), 6565-6574.

Wang, D., Cheng, L., Li, J., Deng, B., Yan, T., Yue, X., . . . Xie, J. J. E. J. o. P. S. (2021). Peptide DR8 analogs alleviate pulmonary fibrosis via suppressing TGF-β1 mediated epithelial-mesenchymal transition and ERK1/2 pathway in vivo and in vitro. 167, 106009.

Wang, D., Yan, Z., Bu, L., An, C., Deng, B., Zhang, J., . . . Pharmacology, A. (2019). Protective effect of peptide DR8 on bleomycin-induced pulmonary fibrosis by regulating the TGF-β/MAPK signaling pathway and oxidative stress. 382, 114703.

Wellbrock, C., & Hurlstone, A. J. B. p. (2010). BRAF as therapeutic target in melanoma. 80(5), 561-567.