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NISIN ZP MODULATES CALCIUM HOMEOSTASIS TO INDUCE APOPTOSIS AND ATTENUATE CELL PROLIFERATION IN MG-63 OSTEOSARCOMA CELLS

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ABSTRACT

Osteosarcoma (OS) is the most common primary malignant bone tumour, characterised by aggressive growth and a high risk of metastasis, particularly in children, adolescents, and the elderly. Despite advances in surgery, chemotherapy, and radiotherapy, treatment outcomes remain unsatisfactory due to therapy-associated toxicity, chemoresistance, and poor long-term survival. These limitations highlight the need for novel therapeutic strategies with greater selectivity and reduced adverse effects. Nisin, a bacterially derived antimicrobial peptide widely used as a food preservative, has recently been investigated for its anticancer properties, with no data on OS. This study evaluated the effects of nisin ZP (NZP) on MG-63 OS cells in vitro, comparing them to 0.46 µg/ml doxorubicin (DOX), the NZP–DOX combination, and NZP with bepridil (BEP), a calcium channel blocker. A comprehensive set of assays was employed, including an MTS cell viability assay, Annexin V/Dead Cell assay via flow cytometry, morphological examination, intracellular calcium concentration measurement, analysis of apoptotic gene and protein expression, and cell cycle assessment. The impact of NZP on normal osteoblasts (hFOB1.19) was also determined to evaluate selectivity. The IC_{50} of NZP in MG-63 cells was at 543.8 µg/ml, which is higher than in other cancer models, suggesting relative cell-specific resistance. NZP significantly reduced proliferation and induced apoptosis in MG-63 cells, while sparing normal osteoblasts, suggesting the potential of nisin as target therapy. These effects correlated with the significant increase of intracellular calcium levels, which were markedly attenuated by BEP co-treatment, supporting the role of calcium signalling in NZP-mediated apoptosis. Gene and protein expression analyses confirmed activation of intrinsic apoptotic pathways, with upregulation of BAX, caspases, and calpains, and downregulation of BCL-2. Furthermore, NZP disrupted cell cycle progression, contributing to growth inhibition. In conclusion, NZP exerts selective cytotoxic effects on OS cells through calcium-dependent apoptotic mechanisms and inhibition of cell cycle progression, providing novel insights into its therapeutic potential. To the best of our knowledge, this is the first study to demonstrate calcium-mediated apoptotic signalling as a key mechanism of NZP action in human OS. Further research on different variants of nisin on other OS cell lines, therapeutic study via 3D culture and using animal models, will strengthen the understanding of the anticancer role of nisin in OS.

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TABLE OF CONTENTS

CHAPTER 1
INTRODUCTION

1.1 Research Background

Osteosarcoma (OS) is a rare cancer despite being the most common bone malignancy, primarily affecting children, adolescents and the elderly. It accounts for approximately 2% of childhood cancer (Jafari et al., 2020; Yin et al., 2024). Its 5-year survival rate is approximately 70% and shows improvement (Prater & McKeon, 2023). OS has high metastatic capacity, predominantly to the lungs, thus posing significant challenges in its treatment (Morya et al., 2025; Zhra et al., 2025).

The treatment options for OS encompass surgery, chemotherapy, radiotherapy, immunotherapy, gene therapy, and targeted therapy. Among these, surgical resection is a primary method for removing tumours, but it is associated with several potential complications, including limb loss, infections and nerve damage (Myers & Chouvin, 2023). These complications can hinder recovery and may necessitate further medical interventions.

Currently, adjuvant chemotherapy, typically involving a combination of methotrexate, doxorubicin, and cisplatin, is a widely preferred treatment approach for OS (Zhang et al., 2020). However, these chemotherapeutic agents are associated with significant adverse effects. For example, doxorubicin is known to cause cardiotoxicity and myelosuppression, which can severely impact patient health and long-term outcomes (Johnson-Arbor & Dubey, 2023).

Radiotherapy is particularly recommended for patients with inoperable tumours. For those who undergo surgery, it can still enhance outcomes, though it can cause complications like radiation pneumonitis and mucositis (Chaput & Regnier, 2021). Immunotherapy, a newer option, modulates the immune system to target cancer cells, but its effectiveness can be unpredictable (Giri et al., 2025). Similarly, gene therapy holds promise but is hindered by ethical concerns, policy-related issues, and financial constraints (Papanikolaou & Bosio, 2021). Therefore, the quest for novel treatments that can induce targeted apoptosis in OS cells is critical to enhance survival rates and mitigate treatment-associated complications and adverse effects in the current treatment options.