UNIVERSITI TEKNOLOGI MARA

TARGETING CHEMORESISTANCE IN OSTEOSARCOMA: EVALUATING THE EFFICACY OF THYMOQUINONE NANOPARTICLES AND HYPERTHERMIA IN 3D CELL CULTURE OF OSTEOSARCOMA MG-63

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ABSTRACT

Osteosarcoma is a highly aggressive bone cancer with a poor prognosis due to its high metastatic potential and resistance to conventional therapies. The main reason for this resistance is the presence of cancer stem cells (CSCs), which exhibit strong DNA repair abilities (via MutS Homolog 2 (MSH2) overexpression) and self-renewal capacity (regulated by NANOG), allowing them to evade chemotherapy-induced apoptosis via up-regulation of Cysteine-aspartic acid protease 3 (Caspase-3). CSCs often exist in low-oxygen (hypoxic) areas of tumors, which limits drug delivery and reduces treatment effectiveness. As a result, standard therapies usually fail to eradicate all CSCs which led to tumor relapse and disease progression. To overcome this, this study explores using thymoquinone nanoparticles (TNP) combined with hyperthermia (HT) to make CSCs more responsive to treatment. Specifically, this study aims to: (i) develop a 3D co-culture spheroid model incorporating human fetal osteoblasts (hFOB 1.19), osteosarcoma (MG-63) cells, and CSCs, (ii) determine the viability and proliferation of spheroids following first and second cyclic treatments of TNP and HT, and (iii) assess the impact of MSH2, NANOG, Caspase-3 and Heat Shock Protein 70 (HSP70) expression to evaluate their roles in DNA repair, self-renewal, and apoptosis and further investigating how HSP70 contributes to cellular stress responses and survival mechanisms within the spheroid microenvironment under therapeutic stress conditions. The 3D spheroids were treated with TNP and HT in two cyclic treatments, followed by recovery phases. The combination of TNP and HT significantly reduced CSC viability and growth. After the second treatment cycle, MSH2 expression dropped by –3.06-fold, showing reduced DNA repair ability. NANOG decreased initially (–2.21-fold) but increased later (2.12-fold), suggesting CSC adaptation. Caspase-3, an apoptosis marker, increased in the first cycle (1.42-fold) but decreased in the second (–0.62-fold). HSP70, related to stress resistance, also declined (–0.72-fold). In co-culture spheroids, treatment effects were stronger which resulted in Caspase-3 increased (1.38-fold), NANOG decreased (–0.84-fold), and HSP70 showed a larger drop (–1.73-fold). These results suggest that cell–cell interactions in co-culture enhanced the treatment response. Overall, TNP and HT effectively reduced CSC chemoresistance by targeting DNA repair, self-renewal, and survival pathways, especially in co-culture models with minimal effect on hFOB 1.19. These findings highlight a promising therapeutic strategy to overcome chemoresistance and improve osteosarcoma treatment outcomes.

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CHAPTER 1
INTRODUCTION

1.1. Research Background

Osteosarcoma is a highly aggressive primary bone malignancy that predominantly affects the long bones of children and adolescents (Beird et al., 2022). Despite the availability of treatments such as surgery, chemotherapy, and radiotherapy, survival outcomes remain suboptimal due to tumor heterogeneity and intrinsic resistance to therapy (Jiang et al., 2022). The five-year survival rate for localized osteosarcoma is approximately 52%, but it falls drastically to 22% in metastatic cases (Ando et al., 2013; Li et al., 2016). Moreover, the Malaysian National Cancer Registry has reported a 5.7% increase in bone cancer incidence, underlining the need for more effective therapeutic strategies (Azizah et al., 2019).

A major challenge in osteosarcoma treatment is chemoresistance, primarily cause by a subpopulation of cancer stem cells (CSCs) (Garcia-Ortega et al., 2022). These CSCs possess enhanced capabilities for self-renewal, differentiation, and resistance to apoptosis and therapy (Marquardt et al., 2018). Key molecular mechanisms include overexpression of DNA repair enzymes like This study investigated the combined effects of thymoquinone nanoparticles (TNP) and hyperthermia (HT) on osteosarcoma cancer stem cells (CSCs) and co-culture spheroids, focusing on their impact on chemoresistance mechanisms. Given that CSCs play a crucial role in osteosarcoma recurrence and resistance to conventional therapies, targeting their survival mechanisms is essential for improving treatment outcomes. The findings from this study provide significant insights into the potential of nanoparticle-based HT therapy in disrupting CSC-driven resistance with minimal cytotoxicity effects to normal cells (hFOB 1.19).

MutS Homolog 2 (MSH2), a key DNA mismatch repair protein, and stemness-associated transcription factors such as NANOG which plays a pivotal role in maintaining stem cell pluripotency and self-renewal along with Sex-determining Region Y-box 2 (Sox2) and Octamer-binding transcription factor 4 (Oct4), are essential regulators of genomic stability and stem cell characteristics. These factors collectively support CSC survival and contribute to tumor recurrence and metastasis (Jentzsch et al., 2014; Martins-Neves et al., 2022). Furthermore, CSCs down-regulate pro-apoptotic