UNIVERSITI TEKNOLOGI MARA

INVESTIGATION OF THE INTEGRIN GLYCOSYLATION INHIBITION ON LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 8 (LRP8) EXPRESSION THROUGH WNT PATHWAY IN OSTEOSARCOMA (OS) CELL LINE

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ABSTRACT

Osteosarcoma (OS) is the most common malignant bone tumour with bimodal age distribution, affecting children, adolescents and those aged 60 and older. The 5-year survival rate has increased significantly with effective treatment management (60-70%), yet it has decreased drastically for metastasised OS patients (30%). Additionally, aberrant glycosylated integrins was associated with tumour malignancy in melanoma and ovarian cancers. Moreover, WNT/β-catenin pathway was reported as a potential underlying pathway in facilitating OS metastasis where Low-density lipoprotein Receptor-related Protein 8 (LRP8) revealed to act as a positive regulator in canonical WNT/β-catenin signalling pathway. Thus, this work intends to firstly determine the role of integrin glycosylation in OS cell adhesion and migration via extracellular matrix (ECM) proteins, and secondly to investigate the role of LRP8 in WNT/β-catenin signalling pathway in OS progression post integrin glycosylation inhibition and LRP8 knockdown. Briefly, hFOB 1.19, MG63 and HOS 143B cells were treated with 0.5 mM of glycosylation inhibitor; 1-DNJ for 24 hours. Glycosylated proteins of treated hFOB 1.19 and MG63 were determined, ECM-cell adhesion of treated MG63 was observed, migration rates of hFOB 1.19, MG63 and HOS 143B were determined and quantified, and ECM protein expressions (FN, VN and col II) were determined via western blot (WB). The second part of the study entailed the optimisation for optimum time point of LRP8 shRNA transduction in hFOB 1.19, MG63 and HOS 143B cells. LRP8 knockdown was chosen at MOI 5 (hFOB 1.19) and 10 (MG63 and HOS 143B) respectively for 72 hours. Successful LRP8 knockdown was observed by GFP signals, and its expression was measured by RT-PCR and WB post 24-hour of 1-DNJ treatment. LRP8, β-catenin and AXIN2 expressions were determined post 1-DNJ and post 1-DNJ LRP8 knockdown via RT-PCR and WB. LRP8 gene was reduced significantly post LRP8 knockdown ($p<0.05$) at 72 hours for all cells; hFOB 1.19, MG63 and HOS 143B. Next, LRP8 gene was upregulated in post 1-DNJ LPR8 knockdown in both MG63 and hFOB 1.19 ($p<0.05$) cells compared to their post 1-DNJ groups while LRP8 was downregulated in HOS post 1-DNJ LPR8 knockdown. β-catenin expressions were decreased in both MG63 and hFOB 1.19 in post 1-DNJ LRP8 knockdown groups compared to their post 1-DNJ groups while slightly upregulated in HOS 143B post 1-DNJ LRP8 knockdown compared to its post 1-DNJ group. Meanwhile, AXIN2 expression in MG63 post 1-DNJ LRP8 knockdown was upregulated compared to both hFOB 1.19 and HOS 143B. In conclusion, integrin glycosylation inhibition by 1-DNJ and the LRP8 knockdown in OS cells was the first study in our knowledge to be utilised together which resulted in increased adhesion and migration rates via ECM proteins and involvement of LRP8 in the canonical WNT/β-catenin signalling pathway. The findings of this study are expected to have significant implications for the future research particularly in investigating the role of glycosylation and LRP8 gene expressions in the progression of OS metastasis and could hold potential value for the advancement of therapeutic treatment targeting the OS metastasis progression.

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'Cause all of the stars, are fading away,
Just try not to worry, you'll see them someday,
Take what you need, and be on your way,
And stop crying your heart out - Oasis.

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CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Osteosarcoma (OS) is the most prevalent malignant bone tumour that occurs in a bimodal distribution patterns, commonly in the first peak of life (children and adolescents) and also later in life (at 60 years old and above) (Rojas et al., 2021). OS typically affects the long bones, femur, tibia, and humerus and with small occurrences in jaw and heart. Current treatment management for OS includes tumour resection via surgeries, combination of adjuvant chemotherapy and radiotherapy. With these current multidisciplinary treatments, the 5-year overall survival rate has risen to 70% in the localised disease setting. However, patients with metastasis tend to have poor prognosis (20 to 30% of 5-year survival rate) and chemo-resistance. Furthermore, its high invasion rate destroys the surrounding bone and soft tissues, and in more than 90% of cases (Ab-Rahim et al., 2015). Therefore, understanding on the cell signalling pathway in osteosarcoma genesis, adhesion, migration, and metastasis is crucial towards a better prognosis for OS.

Metastasis is a multistep process which only involves a certain cell within a heterogeneous tumour population. The hallmark of metastasis are the detachment of epithelial cells from the extracellular matrix (ECM), survival within the bloodstream and growth at the metastatic site (Ray & Jablons, 2010). Cell typically interacts with ECM through various adhesion receptors, including integrins. Integrins are a major family of cell to cell and cell to ECM adhesion proteins. There are two subunits in integrins: subunit α and subunit β. Beside their adhesive functions, integrin also involved in intracellular signalling and in regulation of cytoskeletal formation. In addition, integrins played role in cell adhesion and migration and has been reported to increase the metastasis potential in U2OS OS cell line (Nguyen et al., 2016). Previous study has reported that the upregulation of integrin in OS has been linked to physiological differentiation of cells and pathological processes such as tumour metastasis (Holle et al., 2016).

There are many examples demonstrating that integrin function depends on its glycosylation and that variant glycosylation may be a regulatory mechanism for β_1