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THE ENRICHMENT OF BLADDER CANCER STEM CELLS USING THREE-DIMENSIONAL (3D) SPHEROID CULTURE SYSTEM

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ABSTRACT

Bladder cancer is a malignancy with a very high rate of relapse. In addition, the presence of tumour-initiating cells also known as cancer stem cells are resistant to conventional treatment modalities. This study analysed methods to effectively enrich the CSCs population from two bladder cancer cell lines, 5637 and HT-1376 using two types of spheroid culture methods; multicellular spheroid (MCS) and single-cell derived spheroid (SCDS). Determination of spheroid formation potential, the expression of CSC surface markers (CD24/CD44, CD44/CD133, and CD133/CD24), pluripotent genes (SOX2, NANOG, and POU5F1), and differentiation potential were analysed in both spheroid cultures. Additionally, MCS and SCDS resistance towards cisplatin treatment and the expression of drug efflux gene (ABCG2) were determined by treating MCS and SCDS cultures with the IC_{50} dosage of cisplatin that was determined earlier in parental 5637 and HT-1376 and spheroid diameters were recorded. Results show that 5637 and HT-1376 could generate spheroids of various structures and cell-cell adhesion strength when subjected to MCS and SCDS culture methods. Spheroid diameters were significantly increased at day 10 post-culture compared to day 2 of MCS of 5637 ($p<0.0001$) and HT-1376 ($p<0.001$) and SCDS of 5637 ($p<0.0001$) and HT-1376 ($p<0.0001$). 5637 MCS produced higher population of CD133/CD24 ($p<0.001$) and CD24/CD44 ($p<0.0001$), while 5637 SCDS generated higher population of CD24/CD44 only ($p<0.0001$) when compared to parental cells. Meanwhile, HT-1376 MCS showed higher population of CD133/24 ($p<0.001$) and CD24/44 cells ($p<0.0001$), while HT-1376 SCDS showed higher population of cells expressing all the 3 surface markers combination, CD133/CD24 ($p<0.001$), CD24/CD44 ($p<0.0001$) and CD44/CD133 ($p<0.0001$) when compared to parental cells. 5637 SCDS expressed higher SOX2 ($p<0.01$), NANOG ($p<0.0001$), and POU5F1 ($p<0.0001$), while 5637 MCS expressed higher NANOG ($p<0.001$) and POU5F1 ($p<0.001$) only. Whereas both MCS and SCDS of HT-1376 demonstrated higher expression of all 3 pluripotent genes ($p<0.0001$) compared to parental cells. However, both cell lines showed no significant changes in differentiation potential following MCS and SCDS culture. HT-1376 exhibited higher IC_{50} compared to 5637 when subjected to treatment for 48 hours (2.61 µM and 1.15 µM, respectively) and 72 hours (7.00 µM and 4.20 µM, respectively). A significant increase in spheroid diameter of 5637 SCDS ($p<0.001$) and ABCG2 upregulation ($p<0.0001$) following cisplatin IC_{50} dosage treatment demonstrated that cisplatin resistance is associated with spheroid formation. However, HT-1376 SCDS showed no significant diameter change in parallel to ABCG2 downregulation when compared to parental ($p<0.0001$) and MCS ($p<0.0001$). In contrast, HT-1376 MCS exhibited significant diameter reduction before and after IC_{50} dosage treatment ($p<0.05$) although there was a significant ABCG2 upregulation when compared to parental cells ($p<0.05$) and SCDS ($p<0.0001$). However, there was no significant diameter change in 5637 MCS. To conclude, both the MCS and SCDS techniques significantly increased the population of bladder CSCs, however, the SCDS technique revealed a greater elevation of CSC markers and pluripotent gene expression. This study provides insight into the capability of spheroid culture to enrich the CSC population in bladder cancer cell lines. The MCS and SCDS culture methods can be used as models for CSC enrichment, cancer cell behaviour research, disease modelling, and personalised treatment research.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Bladder cancer continues to pose a significant threat to the progress of medical science and healthcare according to the Global Cancer Observatory of the World Health Organisation, bladder cancer is one of the ten most prevalent cancers in terms of incidence rate in 2020, the sixth most prevalent cancer in men, and the seventh leading cause of cancer-related mortality in men worldwide (Sung et al., 2021). Bladder cancer (BC) or urothelial carcinoma (UC) has the highest per-patient total cost from diagnosis to death compared to breast, colorectal, lung, and prostate cancers (Botteman et al., 2003).

Recurrence or relapse of cancer refers to the return of the disease, either at the original site of the primary tumour or at a distal site via metastasis. In the case of bladder carcinoma, the recurrence rate is between 40 and 50 per cent (Yu et al., 2012a), making it challenging to completely eradicate the cancer from the body and exponentially increasing the cost of treatment.

Cisplatin, a metal-based antitumor medication, has been discovered to be effective in treating cancers, including bladder cancer (Dasari & Tchounwou. 2014; Schardt et al., 2019). It is a well-known cytotoxic drug that is believed to be able to interfere with DNA activity and prevent DNA repair once it enters the cell nucleus, ultimately leading to cell death (Dasari & Tchounwou. 2014; Schardt et al., 2019). It has been observed that co-treatment of cisplatin with other potential anticancer drugs (Gemcitabine, Methotrexate, Vinblastine, Doxorubicin, Bleomycin, etc) induces apoptosis or autophagy in a variety of cancer cells, increases the drug effectiveness by increasing anti-proliferative ability, drug uptakes and sensitivity towards the drug (Dasari & Tchounwou. 2014).

Multiple factors, such as the grade and stage of the cancer, as well as external factors such as the patient's lifestyle, influence the likelihood of a cancer recurrence. Several studies agree, however, that a type of cell that makes up a small portion of the tumour bulk called the cancer stem cell is also responsible for cancer recurrence (Yadav