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Data sheet of genotoxicity tests for designated food additives in Japan, conducted by the ministry of health, labour and welfare

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ABSTRACT— In this study, tumorspheres were generated from TW06 nasopharyngeal carcinoma cell line and examined their expression of putative cancer stem-like cell surface markers and drug sensitivity. The rate of tumorsphere expansion from dissociated late passage TW06 tumorspheres (≥ passage 15) was higher than that from parental cells and dissociated 10-day-old (passage 0) tumorspheres. The expression of CD24 surface marker was lost in the generation of tumorspheres and the loss was reversible after differentiating the tumorspheres in monolayer culture conditions. Drug sensitivity assay showed that late passage tumorspheres were resistant to docetaxel and oxaliplatin treatment. Our data suggest that serially passaged tumorspheres possess the characteristics of CSCs that render them a suitable preclinical in vitro model for evaluating anticancer drug efficacy and elucidating the underlying mechanisms of drug resistance.

KEYWORDS: Tumorspheres, Cancer stem cells, Nasopharyngeal carcinoma, Chemoresistance.

1. INTRODUCTION

Tumor recurrence and metastasis are two of the major obstacles in cancer treatment which results in mortality. Cancer stem-like cells (CSCs) are widely linked to tumorigenesis and metastasis [1- 3]. In addition, these cells have also been proposed to be linked to chemoresistance and also resistance to radiotherapy [4- 8]. According to the CSC model, tumor is initiated by a subpopulation of cancer cells termed as cancer stem-like cells. These CSCs have intrinsic properties that are identical to normal stem cells which includes longevity and self-renewing ability. Normal adult tissues have a small portion of stem cells that play a role in the replacement of terminally differentiated cells. During self-renewal, these stem cells generate an identical stem cell and also a progenitor cell which will further give rise to a number of differentiated cells. Similarly, these cancer stem cells have the ability to initiate tumor in immune-deficient mice. CSCs were first identified in leukemia and later found in a wide variety of solid tumors. One of the methods that is frequently used as a way to maintain these CSCs in vitro is to culture them in anchorage-independent conditions as tumorspheres [9]. This culture method is originally established from a neural cell activity assay [10]. This method has been adapted into many other studies that are linked to CSCs. Nasopharyngeal carcinoma occurs more frequently in regions of South East Asia [11]. Most of the mortality in NPC patients is believed to be due to distant metastasis and local recurrence of the cancer [12]. Chemotherapies have been developed based on the ability of these chemotherapeutic agents to cause regression of tumor. Cisplatin combined with 5-fluorouracil has been widely used as a standard regimen for metastatic NPC [13- 15]. However, cisplatin-based chemotherapy are often associated with increased and acute toxicities. Newer agents such as taxanes, gemcitabine and capecitabine exert more effective antineoplastic activities in both NPC and other head and neck cancers [16- 18]. Docetaxel is a member of the taxane drug class which shows activity against a variety of solid tumors including breast, lung and

squamous cell head and neck cancers. The ability of these drugs in NPC has been studied in combination with platinum drugs in both metastatic/recurrent and locally advance cancer [19], [20]. Oxaliplatin (OXA), on the other hand, blocks DNA replication and transcription by forming intrastrand cross-links in DNA. Resistance towards chemotherapeutic agent which is one of the characteristics of CSC, is thought to be one of the main causes of cancer recurrence. Although many other theories have attempted to explain chemoresistance, the CSC theory has attracted much interest. Since CSCs are believed to be only less than 10% of the total tumor population, tumor regression by chemotherapeutic drug is expected to be mainly due to the elimination of the non-CSC population. This allows CSCs to remain after chemotherapy and they are able to regenerate the tumor causing tumor recurrence. CSCs can also be progenitor cells in the bulk tumor by going through self-renewal and cell division which causes metastasis.

2. DISCUSSION

Culturing cells in low adherent culture allows them to form cell aggregates termed tumorspheres. Tumorsphere culture has been very popular in the study of CSCs as it has been reported to enrich the CSC population in many cell lines such as breast, liver, colon and ovarian cancer cell line [22]. Under serum-free condition, the CSCs can be maintained in an undifferentiated state. These tumorspheres were maintained in serum-free media with the addition of various growth factors. In this study, the CSC enrichment by tumorsphere culture of a nasopharyngeal carcinoma cell line, TW06 was carried out. The cell line was able to form viable tumorspheres after 10 days of culture in non-anchorage condition. The CSC hypothesis states that tumorigenesis is initiated by cells with stem cell-like characteristics which have acquired a proliferative potential and have ability to self-renew. Our experiment showed that the late passage tumorspheres of TW06 showed the highest expansion rate between day 3 and day 9 in culture as compared to the parental cells and passage 0 tumorspheres (Table 3). This observation was consistent with what some authors reported [23- 25] where CSC had shown rapid proliferation rate as compared to non-CSC. Using cell surface markers is ideal for isolation and identification of CSCs, if more specific markers were identified. Currently, inspecific markers have been identified in various tumors [26- 29]. As for nasopharyngeal carcinoma, [30] had reported that $CD24^+$ cells isolated from TW02 and TW04 nasopharyngeal carcinoma cell lines have CSC-like properties such as increased expression of stem cell genes, enhanced proliferation and sphere formation, and also the ability to induce tumor in NOD/SCID mice. In another study, $CD44^+$ cells isolated from nasopharyngeal carcinoma cell lines, SUNE-1, C666-1 have CSC-like properties [31], [32]. These studies suggested that CD24 and CD44 may be regarded as a marker of CSCs of nasopharyngeal carcinoma. However, our study showed loss of $CD24^+$ population during/in the generation of tumorspheres from TW06 cells which are ~92% positive for CD24 (Table 1). The CD24+ population was increased after differentiation (Table 2). This data is in concordance with an earlier finding by Hermann et al. who found that $CD133^+$ subpopulation from human pancreatic cell line which have CSC-like properties were able to differentiate into $CD133^-$ tumor cells which were non-tumorigenic [33]. Tumorigenicity of these tumorspheres in NOD/SCID mice needs to be further investigated.

3. CONCLUSIONS

In conclusion, the late passage tumorspheres of TW06 NPC cell line has higher expansion rate than its parental cells. Also, the generation of these tumorspheres causes a loss of CD24 expression which is reversible after differentiating the tumorspheres in monolayer culture conditions. Data also suggest that late passage tumorsphere of TW06 NPC cell line consist of enriched numbers of CSCs, which potentially makes them less susceptible to the actions of both DTX and OXA. Further knowledge of these NPC tumorspheres and the mechanism of their resistant to DTX and OXA may provide important information that leads to the development of novel therapeutic strategy for NPC treatment.

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