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Research Article

Selected Conventional Chemotherapy Promotes Cancer Stem Cells in Liver Cancer

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Abstract

Hepatocellular carcinoma is one among the leading cause of death in worldwide. The success rates of chemotherapeutic drugs are very minimal for hepatocellular carcinoma because of multiple reasons. Cancer stem cell (CSC), a small population in tumour play foremost role in tumour relapse and drug resistant through ATP binding caste efflux, ALDH1 inhibition and other unknown mechanisms. This study is designed to identify and evaluate the following standard of care for liver cancer such as Cisplatin, 5-flurouracil, and Paclitaxel, were promote any cancer stem cell like phenotypes or not in HepG2cell lines.

HepG2 cell line was cultured with cisplatin, 5-FU, and paclitaxel in lower level of maximum concentration of serum (Cmax) for three days. Then the cancer stem cell population was evaluated by flow cytometry analysis using cancer stem cell markers such as CD133, and spheroid formation assay.

The expression level of cancer stem cell marker CD133 is exceedingly elevated 2.21% in Low dose 5-Flurouracil treated cells, and very minimal expression 0.96% in Low dose Paclitaxel, 0.91% in Low dose cisplatin. Even though in spheroid formation assay all the drug treated cells shown spheroid, in low dose 5-flurouracil treated cells only formed excellent spheroid whereas in low dose cisplatin and low dose paclitaxel fail to form spheroid as 5-flurouracil.

All the chemotherapeutic drugs were promote cancer stem cell phenotype than control but 5-flurouracil expresses more cancer stem cell phenotype than cisplatin and paclitaxel drug treated cells. It's exceedingly evident for more chances of cancer relapse in 5-Flurouracil treated patients than cisplatin and paclitaxel treated patients.

Keywords: Cancer stem cell, Hepatocellular carcinoma, CD133.

INTRODUCTION

Liver is a vital organ present in vertebrates, and it plays more than 400 functions including production of plasma protein, controls homeostasis, glycogen & lipids storage, albumin & bilirubin production, detoxification of xenobiotics and major roles in metabolism etc. Hepatocellular carcinoma is second most common cause of cancer-associated death worldwide. The incidence of HCC has been rapidly increasing last decade, almost 80000 new cases reported every year[1]. Liver cancer ranks as the fifth most common cancer among both male and female. As reported by a study cohort of 213 HCC patients from 1999 to 2005, the incidence of

HCC is higher in men (83.1%) than in women[2]. Major etiologic factors for HCC are chronic viral infections such as hepatitis B & C, factors like chronic alcoholism and metabolic disorders also modestly involved in HCC[3]. Surgery, chemotherapy and radiotherapy are the standard treatment options for HCC [4]. Most of the conventional treatment fails to eradicate the tumor because of the cancer stem cells (CSC) [5].

Since the new concept of cancer stem cells (CSCs) was introduced in late 1990s, it has gradually gained worldwide acceptance and influenced all approaches to cancer research and therapy. The CSCs, which are also accurately called 'tumor-initiating cells', represent a small population of cancer cells, sharing common properties with normal stem cells (SCs), that can initiate new tumors following injection into animal models, while the majority of other cancer cells cannot. The reported fractions of CSCs in tumors vary from 0.1 to 30% depending on the type and the advancement of the cancer [6]. In newly developing hierarchic cancer models, tumors are functionally heterogeneous and contain various types of cells (e.g., macrophages and vascular endothelial cells and so on). Among them, only CSCs have tumorigenic ability[7].

CD133 also had known as prominin 1 is a five-transmembrane single-chain glycoprotein. Many researchers documented that CD133 is potential cancer stem cell marker for varieties of tumour tissue such as gastric carcinoma, lung cancer, liver cancer[8], and colon cancer as well as pancreatic cancer [9]. Research data support that CD133+ liver cancer cells express stem cell features such as cell proliferation, self renew and differentiation whereas CD133- liver cells cannot [10].

Conventional chemotherapies are initially effective in controlling tumor growth yet many patients relapse over time. This is due to the presence of cancer stem cells. Chemoresistance is a complex mechanism, involving various biological pathways. Also, chemoresistance is a major cause of cancer treatment failure. Cancer stem cell (CSC) in solid cancer has recently identified, but its role in solid organ tumour is not clearly documented. However, research data supported that CSC may involve in carcinogenesis, invasion and metastasis, as well as resistance to various form of chemotherapy [11, 12]. 5-flurouracil, cisplatin and paclitaxel are commonly used therapeutic agents in the clinical settings. This study was designed to evaluate the cancer stem cell properties and cancer relapse of cisplatin, 5-flurouracil, and paclitaxel treatment.

Materials and Methods

Reagents:

Cisplatin (Cat#1550), and Paclitaxel (cat #1567), purchased from Bio vision, and 5-flurouracil (cat #F6627) purchased from Sigma. HepG2 cell lines were purchased through National Centre for Cell Science (NCCS), Pune.

Cell culture /Cell proliferation

HepG2 cells were obtained from NCCS, Pune. The cells were cultured in complete RPMI 1640 medium supplemented with ITS, antibacterial and anti antifungal. Initially the cells were cultured in complete RPMI 1640 medium upto 3 passages to get enough cells, than these cells were seeded in 6 well plates, incubated at least for 24 hours, and reached above 80% confluence before chemotherapeutic agent treatment. Half the value of Cmax concentration such as 1.655 μg/ml dose of cisplatin, 8.3 μg/ml doses of 5-FU, and 1.595 μg/ml dose paclitaxel were added to the cells and cultured for 3 days at 37°C in 5% CO_2 Incubator. Drug medium was changed at every alternative day. The image was captured under inverted microscope.

Flow cytometry

PE-conjugated CD133 monoclonal antibody (clone # W6B3) was purchased from BD Bioscience. After 3 days of drug treatment the HepG2 cells were dissociated with 0.25 % trypsin-EDTA (1 mM) (Invitrogen) for 3 min and washed with Calcium and magnesium free dulbecco phosphate buffered saline solution by spinning at 400g for 7 minutes. Then these cells were diluted in 100 μl FACS buffer (PBS containing 1 % fetal calf serum) and then incubated for 1 h at 4 °C in FACS buffer with the corresponding mAb: anti-CD133-PE. Flow cytometry analysis was performed with a BD FACSCanto II flow cytometer (BD Biosciences).

Spheroid formation assay

3D cell culture reagent, matrigel (Cat.no: 354230) was obtained from BD Biosciences and was used to culture liver spheroids. 5 mg/ ml concentration of matrigel was prepared and used for culturing

spheroids. HepG2 cells were treated with low dose cisplatin , paclitaxel, 5-FU for 3 days respectively. Then these cells were harvested and 1000 Cells/96 well plate were incubated at 37 °C degrees with 5 % CO_2 and culture to get optimal spheroid size. Culture medium was refreshed every 2–3 days up to 9 days.

Results

Morphology analysis of cytotoxic effect of chemodrugs

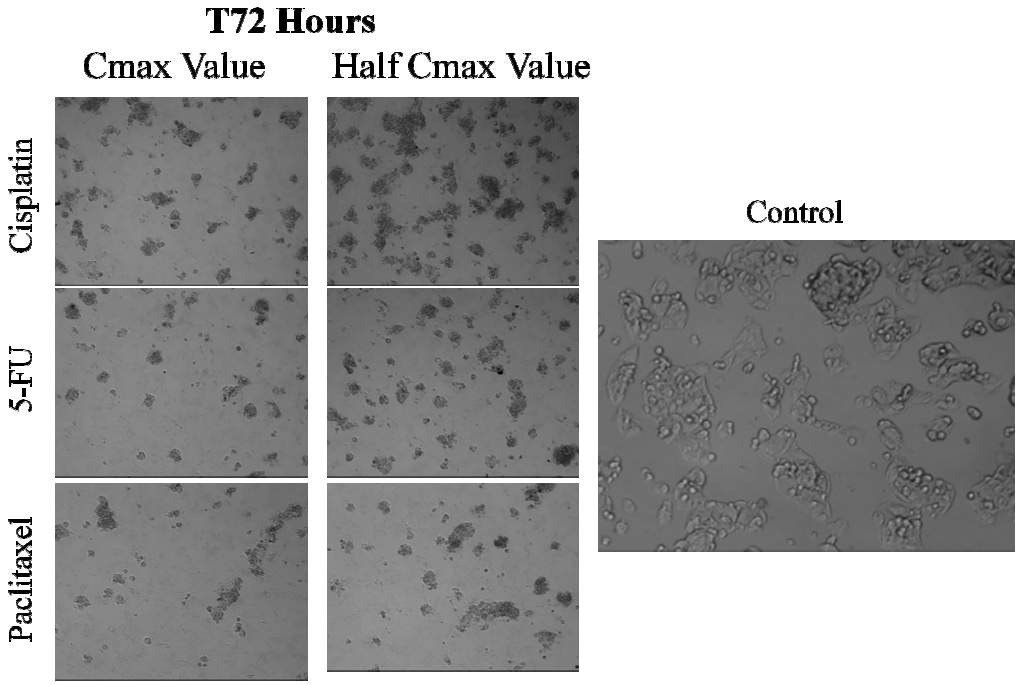

Fig1: Cell death was observed after 3 days of drug treatment HepG2 cell lines. Right side Irregular morphology was observed on Day 3 drug treated cell lines, Left side Cell morphology was maintained after 3 days of culture.

The morphology of control cells remain even cultured after 3 days also whereas in drug treated cells were shrink and lost their morphology. The cell death was observed equally in every concentration of drug treated cells and also there is no much difference shown in cisplatin, 5-FU and Paclitaxel treated cells. It's clearly evident that these drugs shown their cytotoxic effects in maximum concentration of serum level and whatever leftover cells capable of resist the chemotherapy.

Evaluation of cancer stem cell phenotype expression

Part of low dose chemodrugs treated cells were stained for cancer stem cell marker CD133 and analysed in flow cytometry. CD133, a well studied cancer stem cell marker expression was highly enriched at 2.21% in low dose 5-FU treated cells and subsequently the expression pattern was decreased in low dose paclitaxel 0.96% and low dose cisplatin 0.91%. Even though low dose cisplatin and low dose paclitaxel treated cells were significantly expresses the CSC marker it's not as good as low dose 5-fu treated cell expression. It means all the three drugs were promoting cancer stem cells but only low dose 5-fu drug is capable for far above the ground level of chemoresistant and cancer relapse than other two drugs. Notably cell morphology and flow data indicate no matter how much these chemodrugs can kill the cancer cells, it's actually kills only cancer cells but the ultimate cells like CSC escape from the drugs and eventually it 's resistant to the chemodrugs.

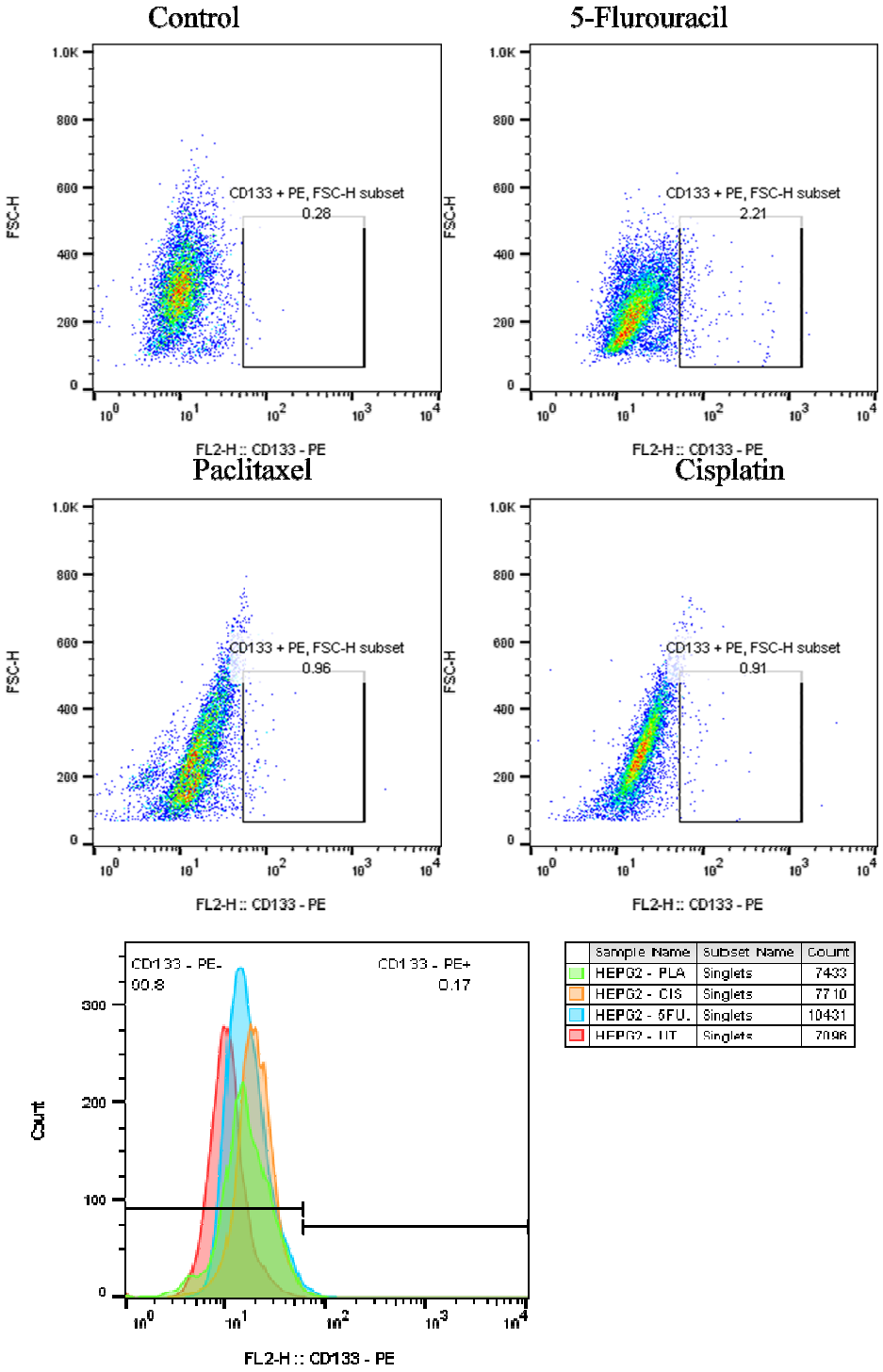

Fig 2: The expression level of cancer stem cell marker CD133 was evaluated by flow cytometry. Only low dose 5-flurouracil treated cells express high level of CD133 followed by cisplatin and paclitaxel than compared to the control. B. The overlay analysis of CSC expression of all three drug treated cells.

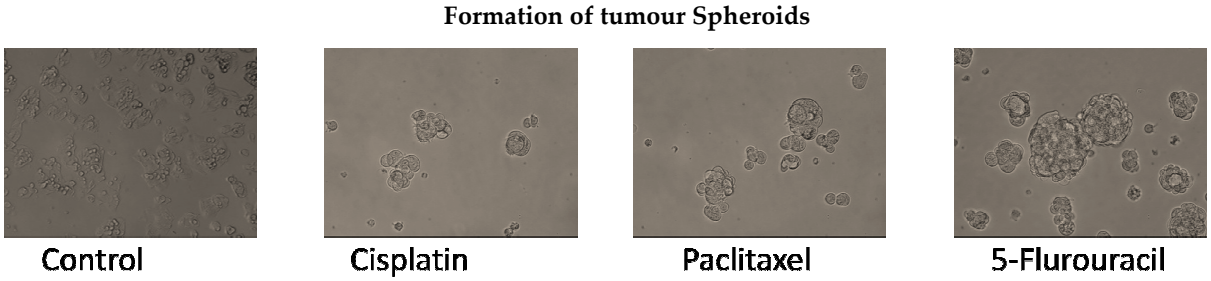

Fig 3: Spheroid formation assay- A well defined spheroid was formed in 5-flurouracil drug treated cells on day 9 of spheroid culture, whereas in cisplatin and paclitaxel treated cells were unable to form spheroid as good as in 5-flurouracil. No spheroid was formed in control even after 9 days of spheroid culture.

A small population of CSC in the tumour can form a spheroid whereas other type of cells was unable to form. Apart from this CSC cells also responsible for chemoresistant and tumour relapse. Normal cells and low dose drug treated cells were used for the spheroid formation assay. After nine day of culture the control cells were unable to form any kind of spheroids while other pre drug treated cells were able to form spheroids in descent size based on the CSC expression.

Discussion

One old theory proposes that a small population of whole tumour named as a cancer stem cells or cancer progenitor cells are responsible for tumourogenisis[13]. Recent studies support that the CSC play major role in outcome of chemotherapy because of their chemoresistance[14]. It was suggested that tumor recurrence might result from the resistance of residual cancer stem cells following chemotherapy[15]. It has also been suggested that tumor stem cells may influence chemotherapy resistance by inducing target related protein mutations, opposing anti-apoptotic processes, and enhancing drug efflux[16]. Ma *et al.* showed that CD133+HCC conformed resistant to the few chemotherapeutic drugs[17]. Our data is great evident that low dose cisplatin, low dose paclitaxel and low dose 5-FU treatement can promote cancer stem cells phenotypes even though it kills the cancer cells. It was confirmed by flow cytometry analysis. In same way through morphological analysis and spheroid formation assay, it is clearly evident that CSC is responsible for chemoresistant and tumour relapse.

Conclusion

No matter this chemodrugs such as cisplatin, paclitaxel and 5-FU have potential target to kill the cancer cells in some mechanism, in another way the same drugs were promoting cancer stem cells in various mechanisms like ATP binding cassette efflux method, ALDH inhibition method and etc. Based on this research, 5-FU has more potential to promote CSC and chemoresistant and tumour relapse than cisplatin and paclitaxel.

Conflict of Interest

The authors declare that they have no conflict of interest.

Reference

1. Tao wang, Lei Xia, Sicong Ma, et al. Hepatocellular carcinoma: thyroid hormone promotes tumorigenicity through inducing cancer stem-like cell self-renewal. Scientific Reports, 2016;| 6:25183
2. Yeole BB. Trends in cancer incidence in esophagus, stomach, colon, rectum and liver in males in India. Asian Pac J Cancer Prev. 2008; 9: 97 -100.
3. Kimura Osamu, Kondo Yasuteru, Kogure Takayuki, et al. Expression of EpCAM increases in the hepatitis B related and the treatment-resistant hepatocellular carcinoma. BioMed Res Int Volume; 2014. Article ID 172913, 8 pages http://dx. doi.org/10.1155/2014/172913.
4. Kane Robert C, Farrell Ann T, Madabushi Rajanikanth, et al. Sorafenib for the

treatment of unresectable hepatocellular carcinoma. Oncol. 2009;14 :95 - 100.

5. Lee TK, Castilho A, Ma S, Ng IO. Liver cancer stem cells: implications for a new therapeutic target. Liver Int. 2009; 29: 955 - 965.
6. Adams JM, Strasser A. Is tumor growth sustained by rare cancer stem cells or dominant clones? Cancer Res. 2008; 68:4018–4021. [PubMed: 18519656]
7. Dick JE. Looking ahead in cancer stem cell research. Nat Biotechnology. 2009; 27:44–46. [PubMed: 19131997]
8. Song W, Li H, Tao K et al. Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma, International Journal of Clinical Practice. 2008; 62: 1212–1218.
1. 9.. Hori Y. Prominin-1 (CD133) reveals new faces of pancreatic progenitor cells and cancer stem cells: current knowledge and therapeutic perspectives. Advances in Experimental Medicine and Biology. 2013; 777: 185–196.
9. Suetsugu A, Nagaki M, Aoki H, Motohashi T, Kunisada T, Moriwaki H. Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. Biochemical and Biophysical Research Communications, 2006; 351: 820– 824.
10. Serguei Vinogradov and Xin Wei, Cancer stem cells and drug resistance: the potential of nanomedicine, Nanomedicine ,2012 ; 7: 597–615.
11. Vasanthakumar S et al. , EpCAM as a novel therapeutic target for hepatocellular carcinoma, Journal of Oncological Sciences, 2017; 3; 71-76.
12. Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. Cell. 2006;124:1111–1115. doi: 10.1016/j.cell.2006.03.011.
13. Lee HE, Kim JH, Kim YJ, Choi SY, Kim SW, Kang E, et al. An increase in cancer stem cell population after primary systemic therapy is a poor prognostic factor in breast cancer. Br J Cancer. 2011;104:1730–1738. doi: 10.1038/bjc.2011.159.
14. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005;5:275–284. doi: 10.1038/nrc1590.
15. Moitra K, Lou H, Dean M. Multidrug efflux pumps and cancer stem cells: insights into multidrug resistance and therapeutic development. Clin Pharmacol Ther. 2011;89:491–502. doi: 10.1038/clpt.2011.14.
16. Ma S, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene. 2008;27:1749–1758. doi: 10.1038/sj.onc.121081.