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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2021.1.3

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2021; 19 (1): 18–22

ORIGINAL PAPER

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The expression of CD44, CD90 and CD133 in response to cisplatin in hepatocellular cancer cells

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ABSTRACT

Introduction. Cancer is a leading cause of mortality. Hepatocellular cancer is one of the malignancies associated with poor outcome and resistance to pharmacotherapy. Cancer stem cells (CSCs) contribute to resistance to therapy and hence lead to the treatment failure of tumors.

Aim. This study aims to explore the expression of CSCs in response to cisplatin treatment in HepG2 hepatocellular cancer cell line.

Material and methods. Cell proliferation test, CCK-8, was used to evaluate the cell proliferation following cisplatin treatment for 72 hours. The expressions of CSC markers CD44, CD90, and CD133 were assessed by flow cytometric analysis.

Results. The results showed a dose-dependent decrease in cell proliferation and increased expression of CSC markers CD44 and CD90 in response to cisplatin.

Conclusion. Understanding the roles of CSC markers may point to new targets and therapeutic strategies to predict and overcome cisplatin resistance.

Keywords. cancer, cisplatin, hepatocellular, stem cells

Introduction

Cancer is a leading cause of mortality despite evolving strategies to treat. The mainstream treatment approach is surgery, radiotherapy, and/or pharmacotherapy. Non- response to pharmacotherapy might be associated with drug resistance, which contributes to failure in the treatment. Drug resistance is a multifactorial phenomenon that involves patient-related factors, tumor-related factors, and surrounding factors.[1] Intrinsic factors or acquired factors during the pharmacotherapy may alter drug response.[2]

Cisplatin (CIS) is the first metal-based antineoplastic drug, which is still one of the most widely used platinum-based anticancer agents in various types of solid cancers.[3] Co-administration of cisplatin with other drugs has clinical importance due to the decreased toxicity and drug resistance.[4]

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 23.11.2020 | Accepted: 14.12.2020
Publication date: March 2021

Domnez Cakıl Y, Ozunal ZG, Kayalı DG, Aktas RG. *The expression of CD44, CD90 and CD133 in response to cisplatin in hepatocellular cancer cells*. Eur J Clin Exp Med. 2021;19(1):18–22. doi: 10.15584/ejcem.2021.1.3

Hepatocellular cancer (HCC), the most frequent type of primary liver cancer, is one of the neoplasms associated with poor outcomes, especially for its non-surgically removable advanced stage. Poor outcome is mainly due to the potential resistance to tumor pharmacotherapy.[5] Therefore, different administration routes are applied to increase the efficacy of the treatment and decrease systemic toxicity. CIS is within the therapeutic approaches with direct hepatic arterial infusion.[6] CIS is still explored for combination therapies with other drugs to ameliorate the efficacy in HCC.

Increasing evidence supports the presence of a small subset of cancer cells with self-renewal and differentiation properties, the so-called cancer stem cells (CSCs). In the liver, CSCs show tumorigenicity and metastasis. Surface molecules; MDR, CD13, CD44, CD45, CD90, CD105, CD133, CD24, EPCAM are linked to CSC traits in HCC.[7,8] CD44, a transmembrane glycoprotein, is the most commonly observed CSC marker.[9] Tumorigenic capacity, an important feature of cancer cells, is associated with CD90 presence in HCC cell lines.[10] Furthermore, cisplatin resistance is highly associated with the biomarker CD133 in various cancers.[11] Overexpression of the CSC markers has been reported to be associated with poorer response to treatment in HCC patients and might have a role in the prediction of drug response.[12]

Aim

This study aimed to investigate the roles of CD44, CD90 and CD133 markers in cisplatin response in HCC by exploring their dose-dependent expression in HepG2 cells.

Material and methods

Cell culture

Human hepatocellular carcinoma cell line HepG2 (American Type Culture Collection) was cultured in 10% fetal bovine serum (PAN-Biotech GmbH, Germany), and 1% antibiotics (streptomycin 10 mg/ml, penicillin 10.000 U/ml, PAN-Biotech GmbH, Germany) containing Dulbecco's Modified Eagle's Medium (Biosera LM-T1720/100, France). Cells were incubated at 37°C in a CO_2 incubator (5%). When they reach 70-80% confluency, they are subcultured with trypsinization.

CCK8 cell proliferation test

The effect of cisplatin (Glentham Life Sciences, UK) on cell proliferation was determined with Cell Counting Kit-8 (CCK8, Abbkine, USA). CIS concentration ranged between 30-4 μM with a 3⁄4 dilution ratio. Cells were incubated for 72 hours after treatments. The optical density of soluble CCK-8 material in each sample is measured with a Synergy Microplate Reader (BioTek, Japan). Each concentration was repeated four times within the plate and three independent experiments were performed.

Determination of cancer stem cell marker expressions by flow cytometry

Following incubation with CIS for 72 hours, drug administered cells and control group were harvested and incubated with BB515 labeled-CD44 (1: 100 dilution), PE-labeled CD133 (1:50 dilution), and APC labeled-CD90 (1:50 dilution) (BD Pharmingen, BD Biosciences, USA). After 30 minutes of incubation at RT, cells were washed with PBS. The pellet was resuspended in PBS and the measurements were carried out in a BD AccuriC6 + flow cytometer (BD Biosciences, USA). At least 20.000 events were collected. The results were analyzed using BD Accuri C6 + software and depicted as dot plots and overlay histograms.

Statistical analysis

All data are the mean of the three independent experiments. CCK8 cell proliferation test results are shown as mean ± standard deviation (SD). Results of CSC marker expression are shown as mean ± standard error of the means (SEM). One-way ANOVA and posthoc Tukey tests were used to identify statistical significance among the groups. GraphPad Prism V.8.2.0 was used for conducting the statistical tests and creating the figures.

Results

Effect of cisplatin on the proliferation of HepG2 cells

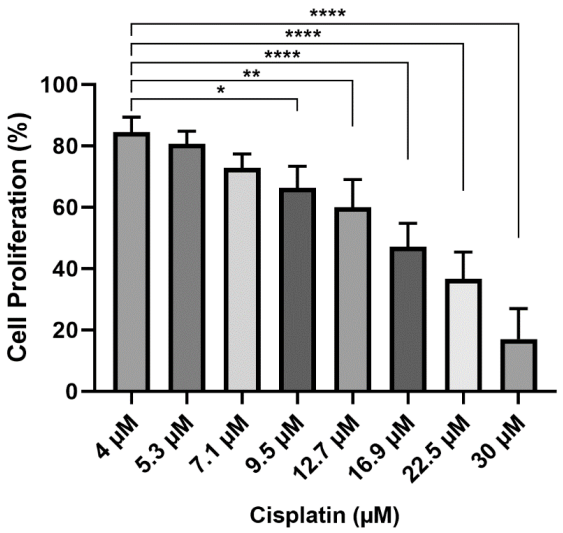

Fig. 1. Effect of treatment with increasing cisplatin doses (μM) for 72 h on HepG2 cell proliferation (%). Statistical evaluation was carried out by one-way ANOVA and post-hoc Tukey analyses were performed. *P<0.05; **P <0.01; ***P< 0.001, ****P< 0.0001 compared with proliferation at 4 μM CIS.

The incubation with decreasing doses of CIS (30-4 μM) for 72 hours exerted antiproliferative effects when compared to the control group (Figure 1). The inhibition was dose-dependent. The effect of the lowest administered

dose, 4 μM, was compared with that of the increasing doses. CIS at 5.3 μM and 7.1 μM did not exert a significant decrease in cell proliferation when compared with CIS at 4 μM. Starting with a dose of 9.5 μM, CIS inhibited cell proliferation significantly in comparison to 4 μM.

Administration of 4 μM, 5.3 μM, and 7.1 μM CIS resulted in 84.5%, 80.6%, 72.9% viability respectively. The highest dose (30 μM) exerted %17 viability.

Effect of cisplatin on CD133, CD44 and CD90 expressions in HepG2 cells

CIS was administered to HepG2 cells at the doses between 7.1 μM and 22.5 μM for 72 hours and the expressions of the CSC markers were analysed by flow cytometry. After treatment with 30 μM CIS, cells were not enough in number for assessing flow cytometric analysis. Representative dot plots for gating of HepG2 cells and CSC marker expressions are given in Figure 2. Representative overlay histograms and bar graphs demonstrating changes in expressions of CSC markers as fold changes are shown in Figure 3.

CD 44 expression increased in response to the cisplatin doses from 7.1 μM to 22.5 μM following incubation for 72 h. The highest CD44 expression was obtained after treatment with 16.9 μM CIS. A more significant increase in comparison to the control group was observed at 12.7 μM and 16.9 μM CIS treatments than 7.1 μM and 9.5 μM CIS treatments.

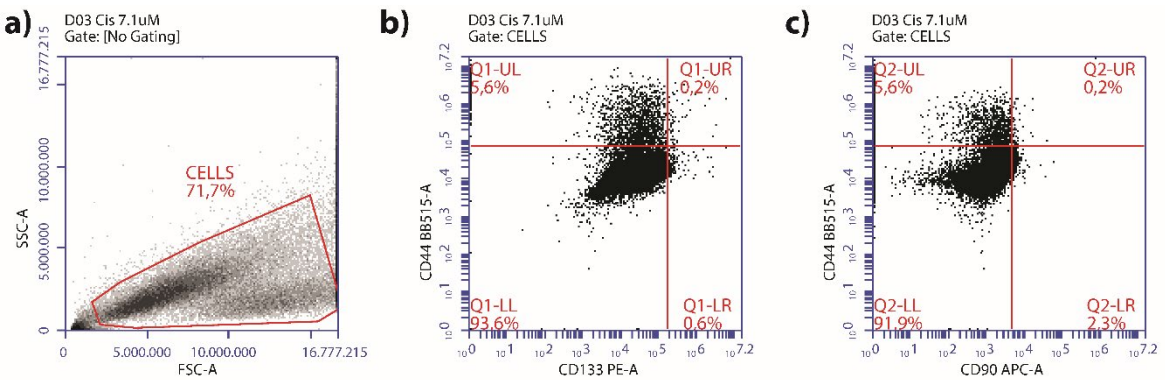

Fig. 2. Representative dot plots illustrate the gating of HepG2 cells (a) and expression of CSC markers (b and c).

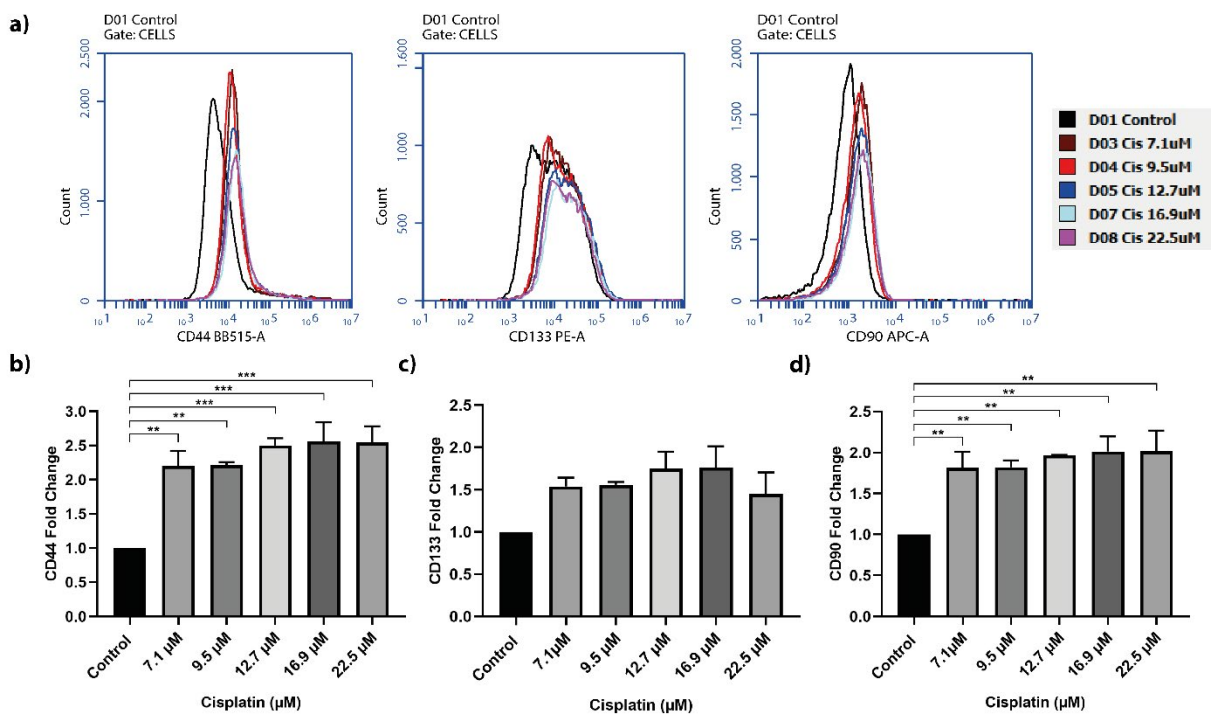

Fig. 3. Representative overlay histograms (a) and bar graphs (b-d) demonstrate the changes in the expressions of CSC markers. Median fluorescence values were obtained with flow cytometric analysis and fold changes were calculated. Statistical evaluation was carried out by one-way ANOVA and post-hoc Tukey analyses were performed. *P<0.05; **P<0.01; ***P<0.001, ****P< 0.0001 compared with control group.

CD 90 expression significantly increased after treatment with CIS at various doses. The groups did not differ significantly and the increase was not dose-dependent.

Unlike CD44 and CD90, the expression of CD133 was comparable with the control group. Although there was an increase in CD133 treatment after CIS treatment, the change was not significant. The effects of the varying doses were comparable with each other.

Discussion

In our study, CIS decreased proliferation in a dose-dependent manner and induced the expressions of CD44 and CD90 but not that of CD133 significantly in hepatocellular cancer cells. These results support that CSCs may play a role in the viability of HCC cells after CIS treatment. CIS was previously shown to increase the fraction of CSCs in head and neck cancer and the researchers suggested the emergence of CSCs as the underlying mechanism for CIS resistance.[13] In hepatoblastoma, a childhood liver cancer, it was clinically shown that increased expression of CSC markers CD44, CD90, and CD133 contributes to reduced survival.[14]

HCC cells demonstrated increased expression of CD44 in response to CIS in a dose-dependent manner. In the lung cancer cells, CIS resistance was decreased with the CD44 knockdown approach.[15] With a parallel aspect, Yin et al. reported that downregulation of CD44 inhibits lung cancer cells and the inhibition is more pronounced when combined with CIS.[16] In another study on HCC cell line, Huh7, the CD44 knockout cells demonstrated that CD44 is involved in the maintenance of CSCs. CD44 seems to be a possible target to overcome CIS resistance also in HCC.[9]

CIS treatment at different doses led to an increase in CD90 expression independent of the dose. Within many CSC markers, CD90 is pronounced as the liver stem cell marker.[10] Wang et al. obtained chemoresistant cancer cells by applying a variety of drugs including cisplatin as single agents or in combination. The researchers showed that CD90 expression increases with drug resistance in PLC, another hepatocellular cancer cell line.[17] Clinically, CD90 expression is significantly associated with rapid recurrence and poor survival in HCC.[18] Moreover, poor response to sorafenib is associated with CD90 overexpression in HCC patients.[12] CD90 is suggested to be a predictor biomarker for therapy.

CD133 expression did not increase significantly following the CIS administration. On the other hand, Zhang et al. induced ALDH1 and CD133 expressions in HepG2 cells using 0-5 μg/mL CIS.[19] Although we observed an increase in CD90 expression, in our study this increase was not found significant. In gastric cancer stem cells, CD133 was found to induce CIS resistance by increasing cell proliferation, anti-apoptosis, and autophagy abilities.[11] In laryngeal cancer, CD133 suppression with curcumin induces CIS sensitivity.[20] Suetsugu et al. examined CSC markers in three hepatocellular cell lines. While HepG2 or Hc cell lines were not stained with anti-CD133 antibody, expression was detected in Huh-7 cells.[21] Our results show the tendency for a change in CD133 expression, yet this trend did not reach statistical significance.

Subpopulations that correspond to CSCs were shown in HCC cell lines including Huh7 and PLC/PRF/5 cells.[22] Cells with CSC properties were detected in several cisplatin resistant cell lines.[23,24] The present study presents increased expression for CSC markers CD44 and CD90 after treatment with cisplatin. Studies on cultured cells isolated from primary tumors would be beneficial to further clarify the role of CSCs in tumorigenicity.

Conclusion

Accumulating evidence prompts the use of the CSCs as an important therapeutic target in HCC. Drug resistance is an important obstacle in pharmacotherapy especially in HCC and exploring drug-resistance related to CSC may lead to new targets. Highlighting the underlying mechanisms is beneficial for the development of novel therapies and might provide a strategy to predict the drug response and overcome the non-response cisplatin treatment.

Acknowledgement

This study was funded by Maltepe University Research Project Council (Grant date 18.02.2019).

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