Cancer Stem Cells (CSCs) formation: Probable explanation for Cisplatin Resistance in Head and Neck Cancers

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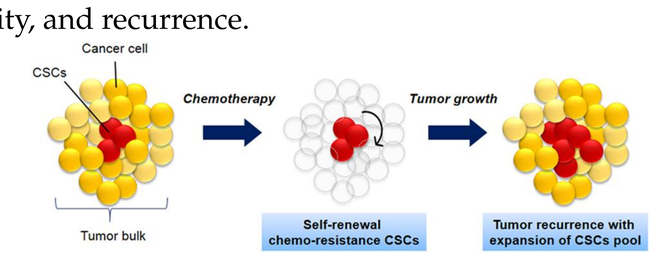
Abstract

Head and neck cancer is the sixth most common cancer worldwide. In India, it comprise approximately 40% of total cancer sites. Cisplatin is one of the most extensively used effective chemodrug for treatment of head and neck cancers. Chronic usage of Cisplatin has irreversible side effects like ototoxicity (hearing loss), neurotoxicity, and nephrotoxicity along with increase risk of secondary malignancies like Leukemia. Also, about 50% patients at some point become non-responsive to it thus allowing reoccurrence or aggressive form of tumor. Hence, it is important to understand the mechanism of Cisplatin resistance. We hypothesize that Cisplatin exposure leads to formation of cancer stem cells which remain non responsive to chemodrug and proliferate.

We tested the effect of Cisplatin on Proliferation potential and Cancer Stem Cells (CSCs) formation of HEp– 2 of laryngeal origin cancer cell line. Higher proportion of Ki-67+ (a nuclear proliferation antigen), CD44+ and Nanog+ (Cancer stem cell markers) cells were found upon treatment with suboptimal dose (IC_{25}) of Cisplatin. This study indicates that Cisplatin resistance occurs due to formation of CSCs. Better understanding of the pathway involved in CSC generation can enable designing of novel therapeutic strategies for non responsive patients.

Introduction

- Head and neck cancer involves 650,000 cases and 330,000 deaths every year.
- A Combination of radiotherapy and chemotherapy can effectively treat 97% of initial tumor phases. However, they have shown to be ineffective for advanced oral cancer.
- Chemoresistance is a major cause of mortality in Head and Neck cancer. Cancer stem cells (CSC) are also reported to be associated with therapeutic resistance of cancer.
- Cancer stem cells (CSCs) are tumor initiating cells, having self-renewal properties and required for maintainance of the cancer. They are also associated with metastasis, tumorigenicity, and recurrence.



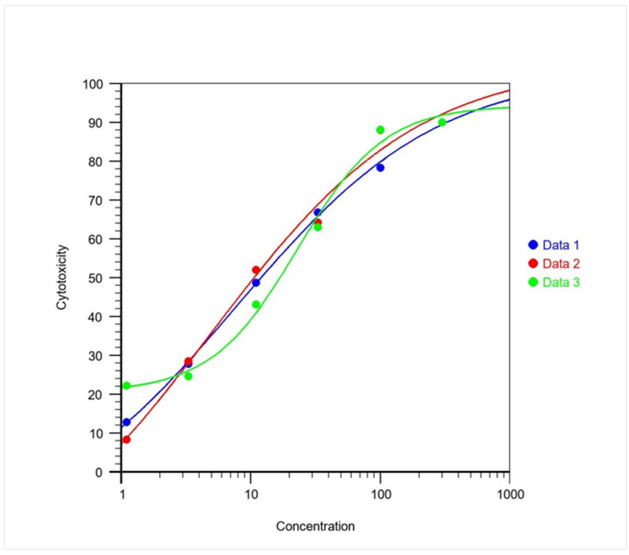
- Cisplatin is commonly used for the treatment of head and neck cancer and is also the most extensively studied chemotherapy agent due to its radio-sensitizing role. It is a platinum analogue which binds to DNA, damages DNA and inhibit DNA repair mechanisms.
- Cisplatin response remains highly variable among individual tumors and development of cisplatin resistance is common. Primary cisplatin-resistance has been regarded as a very poor prognostic factor, a majority of patients develop resistance to cisplatin and die within one year. Understanding mechanisms and effects underlying Cisplatin resistance and developing effective strategies to combat it are highly required to improve tumor reccurence.
- In this study, we have evaluated the effect of Cisplatin treatment on proliferation and CSCs formation of head and neck cancer cells by analyzing the expressions of
  -Proliferation marker Ki-67 (a nuclear protein associated with cell proliferation) and
  -Cancer stem cell markers CD44 (a cell surface glycoprotein) and Nanog (a transcription factor for embryonic stem cell renewal).

Methodology

- **Experimental setup:**
  Human laryngeal origin epithelial cell line – HEp-2
- **Determination of cytotoxic potential of Cisplatin:**
  Resazurin based colorimetric assay was performed to estimate cell viability/death upon treatment with Cisplatin at 72 hours. Percent cytotoxicity was calculated and IC_{25} value was calculated. IC_{25} was considered as an sub optimal dose of Cisplatin and used for subsequent studies.
- **Evaluation of effect of Cisplatin treatment on Proliferation and CSCs formation:**
  Percent of Ki-67+ (a nuclear proliferation antigen), CD44+ and Nanog+ (cancer stem cell markers) was estimated by flow cytometry using fluorochrome conjugated corresponding antibodies. Except CD44, rest of the markers were stained using intracellular staining by fixation and permeabilization.

Results

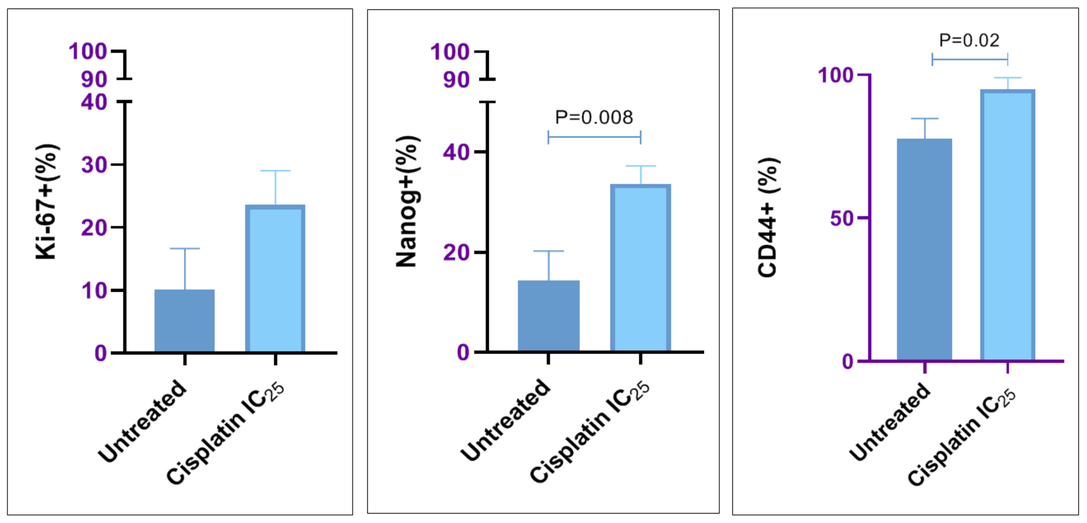
Determination of cytotoxic potential of Cisplatin:



IC_{50} = **13.05 ± 3.31 uM**

IC_{25} = **3.3 ± 0.1 uM**

Evaluation of effect of Cisplatin treatment on Proliferation and CSCs formation:



Discussion and future prospects

- Cisplatin treatment at suboptimal dose, increases proliferation potential and CSCs formation in HEp- 2 cells cancer cell line.
- Understanding the mechanism of CSCs formation can be used to reduce Cisplatin resistance in patients and reduce the associated side effects.

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