INVESTIGATION OF THE ANTI-TUMOR ACTIVITY OF COPPER OXIDE (CUO) NANOPARTICLES

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Introduction

It has been shown that subset of tumor cells termed tumor initiating cells (TICs) possess several features that distinguish them from other tumor cells: They can initiate tumor growth and are considered the subset of cells that promote drug resistance. Therefor, identifying drugs or treatments strategies that can target TICs is a major research effort in cancer biology.

Copper oxide (CuO) nanoparticles (NP) are highly cytotoxic for both cancer cells and TICs[1,2]. The mechanism of action of CuO NP is by inducing oxidative lesions as they produce intracellular reactive oxygen species (ROS)[3,4].

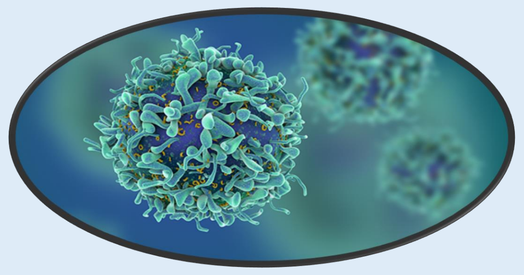
Research hypothesis: CuO NPs can potentially serve as a good agent for treating cancer.

CuO Nanoparticles (NP)

- Their size is between 20–95 nm
- Generate reactive oxygen species (ROS) which causes DNA damage, cell cycle arrest, apoptosis, etc,

VS

Tumor Initiating Cells (TICs)

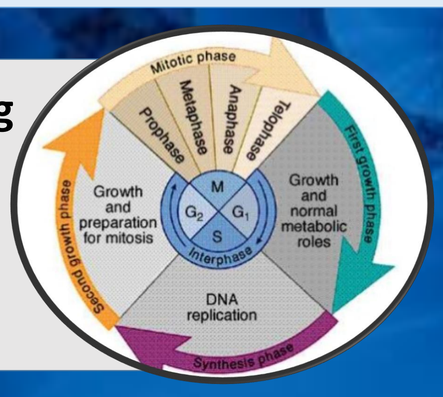


- Capable of self-renewal and undergo differentiation
- Resist to chemotherapy

Methods and Materials

Analysis of cell cycle using Flow cytometer

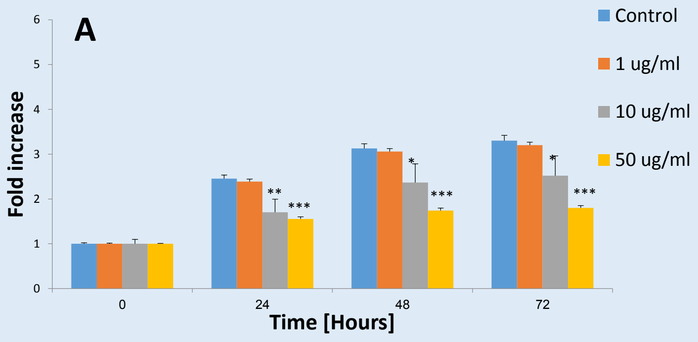
Study the effect CuO NPs on cell cycle of TICs.



CyAn ADP Flow Cytometer

Study the effect CuO NPs on apoptosis, phenotype of TICs, and ROS activity.

1. CuO NPs decrease cell viability significantly in TICs compared to non-TICs cells in dose dependent manner



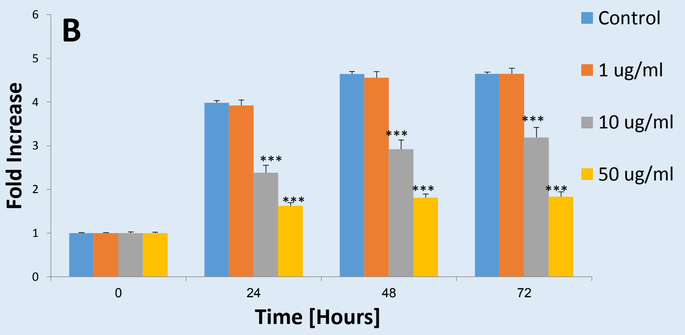


Fig. 1A The effect of CuO NPs on non-TICs and **Fig. 1B** TICs (B) viability was evaluated using AlamarBlue. Cells were cultured in the presence of 1, 10, 50 µg/mL of CO-NP for the designated time points.

2. TICs cell death is increased significantly in a dose-dependent manner

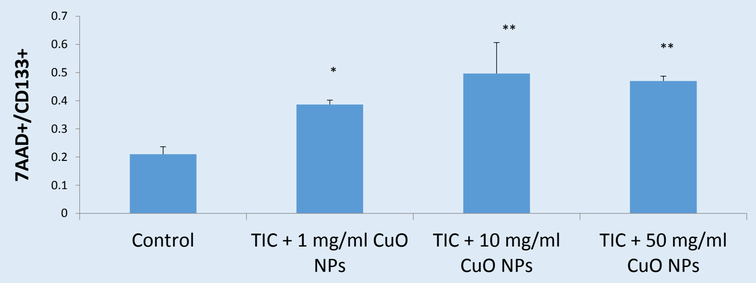
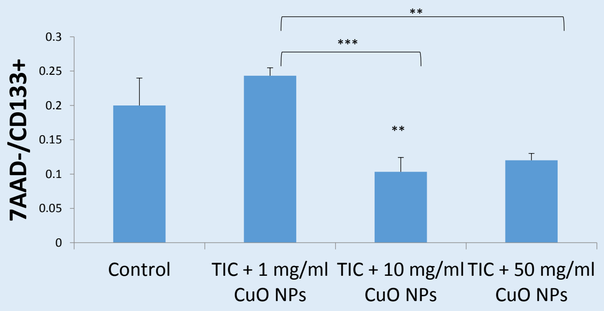


Fig. 2 Evaluation of cell death using 7AAD which is a dye that discriminate between dead and live cells. TICs cells were treated with different concentrations of CuO NPs for 24 hours.

3. CuO NPs decrease TICs CD133⁺ phenotype significantly in a dose-dependent manner



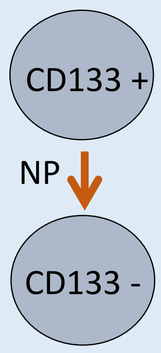


Fig. 3 CD133⁺ was evaluated in TICs with different concentrations of NP.

4. Increase in G2 phase of TICs cells when exposed to higher doses of CuO NP

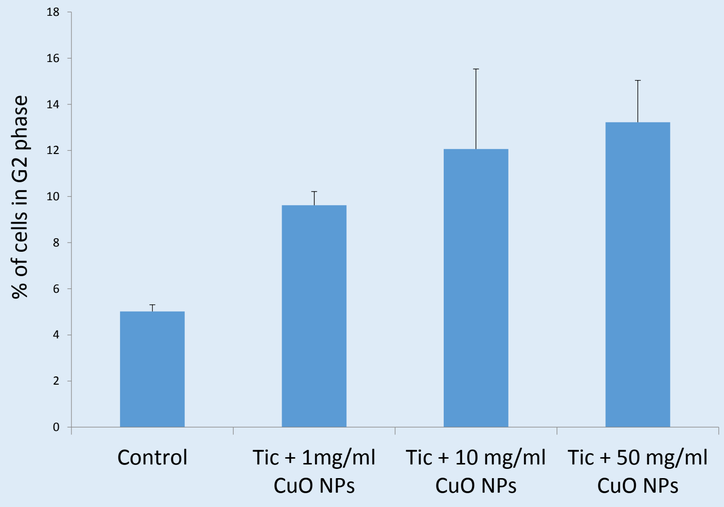


Fig. 4 The cell cycle was checked using Flow Cytometer machine.

G2 phase is a period of rapid cells growth and protein synthesis during which the cell prepares itself for mitosis.

5. CuO NPs induced ROS generation in TICs dose-dependent in TICs

Sample	% of ROS
control	36.73
1 ug/ml CuO NPs	41.72
10 ug/ml CuO NPs	47.88
50 ug/ml CuO NPs	64.21

Fig. 5 Evaluation of oxidative stress induced by CuO NPs.

6. Safety experiment in vivo

Fig. 6 Mice were injected daily with different doses of CuO NPs for one week cycle. Mice weight was measured daily.

Discussion and Conclusions

CuO NPs are potentially a good anti-cancer drug since they induces cell death of TICs by different cellular mechanisms:

- More significant cytotoxic effect on TICs compared to non-TICs (Figs. 1A + 1B).
- CuO NP induction of apoptosis is medicated through ROS production in TICs (Figs. 2 + 5) .
- Cell cycle arrest in G2 phase (Fig. 4) .
- CuO NP causes switch in the CD133⁺ TICs phenotype (Fig. 3) .
- The Nanoparticles were found safe for the concentrations of 5, 20 and 50 µg/kg of CuO NPs (Fig. 6).

References

1. Abdal Dayem A. (2010) "Role of Oxidative Stress in stem, Cancer, and Cancer Stem Cells." Cancer ISSN 2072-6694
2. Benayoun L. and Shaked Y.(2013) "In Vitro Enrichment of Tumor-Initiating Cells from Human Established Cell Lines" Current Protocols in Stem Cell Biology 3.7.1-3.7.15
3. Chang Y.N, Zhang M.Y, et al.(2012) "The Toxic Effects and Mechanisms of CuO and ZnO Nanoparticles" Materials ISSN 1996-1944.
4. Abdal Dayem A., Cho S.G., et.al(2017) "The Role of Reactive Oxygen Species(ROS) in the Biological Activities of Metallic Nanoparticles." Int. J. Molecular Sciences 18010120.

Acknowledgments

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