99P Harnessing copper in cancer to enhance anti-tumor immune response

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Background: Copper is elevated in tumors and the use of copper-chelating agents is under intense investigation. It has been reported that copper plays a key role in the immune system, but its activity is unclear. Recent advances in immunotherapies have shown great potential for treating several cancers. However, tumors express molecules, such as the Programmed Death Ligand 1 (PD-L1), to prevent immune cell activity. Several anti PD-1/PD-L1 therapies have been approved by the FDA, but concerns have been raised about their safety. Therefore, there is a need for different approaches to target this pathway.

Methods: Experiments were performed in 3 neuroblastoma (NB) cell lines SK-N-FI, SH-SY5Y, SK-N-BE(2)C and 2 glioblastoma (GBM) cell lines U87MG, MO59J. PD-L1, T cells markers and Copper transporter1 (CTR1) protein levels were determined using western blot and flow cytometry. Intracellular copper was measured by ICP-MS analysis. In vivo activity of copper-targeting drugs was assessed in NB syngeneic mouse models.

Results: Tissue microarrays from NB and GBM patient tumors showed a significant correlation between CTR1 and PD-L1 expression ($p = 0.00014$ & $p = 0.012$, respectively). Suppression of CTR1 using siRNAs caused a decrease of intracellular copper which in turn led to a downregulation of PD-L1 expression in NB cells, whereas, addition of copper into the media clearly induced PD-L1 mRNA and protein upregulation. Dextran-Catechin (DC) and TEPA, drugs dysregulating copper homeostasis, downregulated PD-L1 expression in both NB and GBM cells. In vivo studies showed that DC prolonged mouse survival by decreasing tumor size in the NB mouse model. Ex vivo immunohistochemistry staining confirmed that downregulation of CTR1 is associated with reduced PD-L1 expression. In addition, DC treatments showed an increase of tumor-infiltrating CD4+ and CD8+ lymphocytes and activated NK cells in the NB immune-competent mouse model.

Conclusions: There is a strong association between PD-L1 expression and intracellular copper levels. Copper dysregulating agents reduce PD-L1 in vitro and in vivo and in turn, promote a significant increase of tumor-infiltrating lymphocytes. This study highlights the potential to enhance tumor immune surveillance by targeting intracellular copper levels.

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