Idronoxil combined with cisplatin rescues refractory immune responses in nasopharyngeal carcinoma

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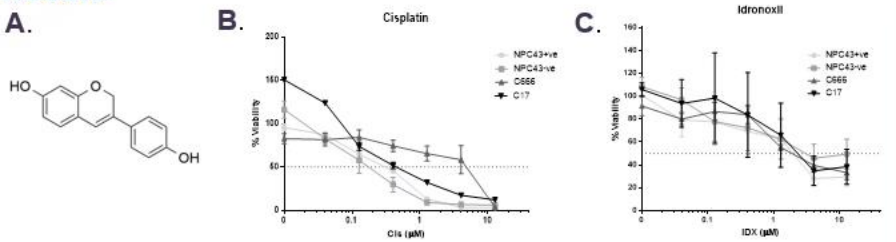
Background:

Nasopharyngeal carcinoma (NPC) is endemic in Southern China. NPC is well-known to be heavily infiltrated by lymphocytes and constitute a unique but poorly defined tumor microenvironment. We aimed at delineating and defining the contribution of idronoxil (IDX), a natural plant-derived isoflavone derivative, in restoring sensitivity to apoptosis and potentially modulating the immune microenvironment of NPC.

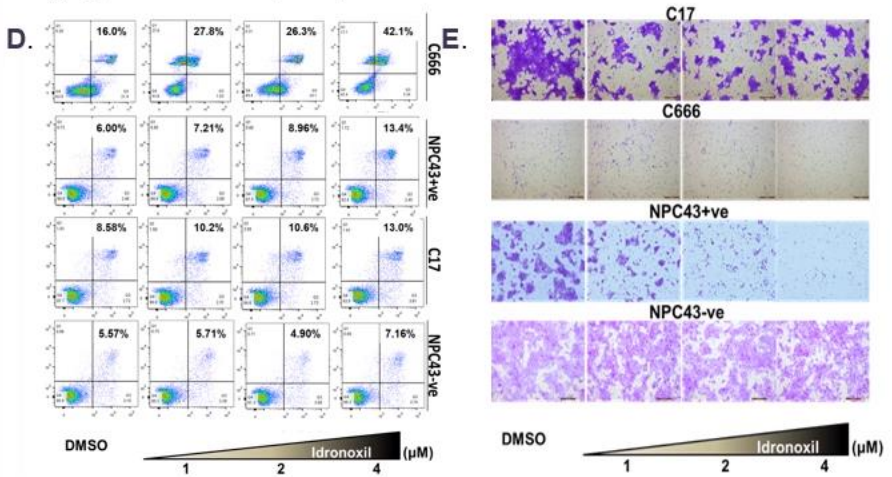
Methods:

- Four authentic EBV-positive NPC-derived cell lines (C666, C17 and NPC43 [EBV-positive+ve and negative-ve}) were used to evaluate IC_{50} by XTT assay;
- Migratory and apopotoic potential of IDX in NPC cells were tested via 8μM-transwell and annexinV/PI flow cytometric staining, respectively.
- HLA-matched PBMC isolated from buffy coat were cocultured with NPC cell lines pre-treated with IDX or combined with cisplatin (cis). After cocultures, PBMC were isolated, washed and stained with antibodies against CD19, CD56, CD14, CD4 and CD8 for immunophenotyping by flow cytometry;
- CFSE-stained PBMCs were cocultured with IDX and/or cis treated NPC cell lines via 5μM transwell and allowed to migrate for 6h. Migrated cells were stained with CD8 antibodies and analysed by flow cytometry.

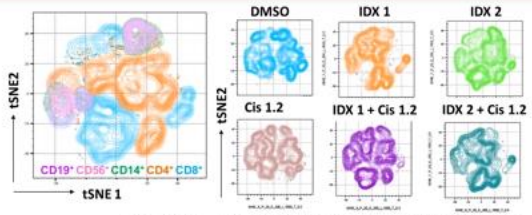
Result:

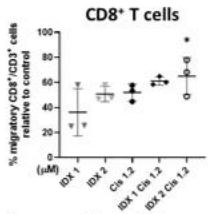


A) IDX chemical structure. B). The viability of cells treated with cisplatin or C) IDX for 5 days was analyzed by XTT cell proliferation assay. The cis demonstrated diverse cytotoxicity with IC_{50} values ranging from 0.2-1.2 μM in the four tested cell lines, while IC_{50} values ranging between 2-4 μM upon IDX treatment..



D) Percentage of Annexin-V/PI positive NPC cells following IDX treatment. E) Representative images of cell migration in 8 μm transwell upon IDX treatment, stained with crystal violet.





F) We evaluated the effects of IDX, cis or IDX-cis combination treatments on immune lineage markers (CD4 T helper cells, CD8 T cytotoxic cells, CD14 monocytes, CD19 B cells and CD56 NK cells) within the cocultures. cis1.2-treated cancer cells showed similar t-SNE coordination in clusters that over-lapped with untreated cancer cells (DMSO). Both numbers of CD4+ and CD8+ subsets were substantially diminished when PBMCs were cocultured with IDX1-treated cancer cells. This effect was restored when cells were treated with IDX2, which was further upregulated upon IDX-cis combined treatment. Similar results were observed for CD8+ T cell migration.

Conclusion:

- IDX modulate T cell populations indicates the drug's potential to enhance the efficacy of current chemotherapy treatments in NPC by upregulating cellular trafficking to the cancer cell;
- IDX shows immune-enhancer activity, in addition to its cytotoxic activities, by targeting tumor-lymphocyte interactions in the tumor microenvironment of NPC.

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