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Evaluation of response to Carboplatin in putative Cancer Stem Cells of Retinoblastoma Y79 cell line

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Abstract

Purpose: Evaluation of Cancer Stem Cells (CSCs) in primary tumors is not only of academic interest but of potential therapeutic application. Due to the technical challenges in working with primary cells, this study attempts to evaluate the response to standard Rb chemotherapeutic agent, Carboplatin in the putative CSC population of Retinoblastoma Y79 cell line. With the preliminary evidence that CD133- FSClo/SSClo cells could be putative Cancer stem cells in Y79 cell line, we evaluated the cytotoxicity of these cells to Carboplatin.

Methods: Phenotypic characterization and sorting of cultured Y79 cells was done by FACS Aria II using putative CSC marker CD133. The sorted cells and total cells were analyzed for response to various doses (0-100µM) of Carboplatin (Alkem Pharmaceuticals) following 48 hr exposure. Controls consisted of untreated cells from CD133± and total Y79 cells. Cell death was observed under phase contrast microscope and the wells were treated with MTT(5mg/ml), the resulting formazan crystals were dissolved with DMSO and absorbance was read at 570nm. A comparative analysis of percentage of viability among the three groups- total Y79 cells, CD133+ and CD133- cells was done using GraphPad Prism .

Results: Retinoblastoma Y79 cell lines when sorted using CD133 revealed 3.8% CD133+ and 16.2% CD133- of total viable Y79 cells. CD133- cells showed increased resistance and proliferation compared to CD133+ and unsorted cells (p<0.01) following 48 hr exposure to higher doses of Carboplatin indicating chemoresistance in this population. Maximum proliferation (151.57±37.54%) was observed at 50µM in

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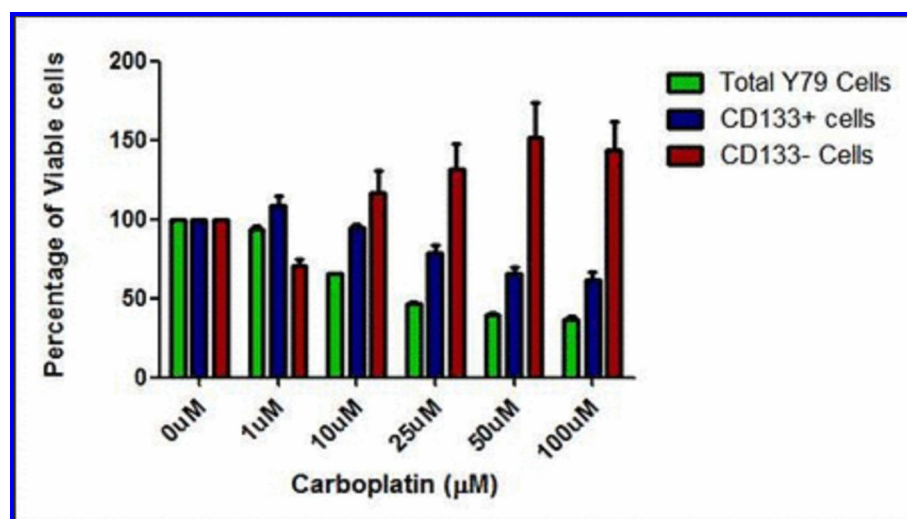
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CD133- cells . At the same dose, the viability of total Y79 cells and CD133+ cells were 39.66 ± 3.05 and 65.22 ± 10.12 respectively.

Conclusions: The study shows that the Y79 CD133- FSClo/SSC1o cells exhibit resistance to high dose Carboplatin with increased proliferation as compared to controls. This is in concordance with our previous findings of quiescence, clonal nature and gene expression (ARVO abstract no 2643/A450) in putative Cancer Stem Cells of Retinoblastoma Y79 cell line.



Drug response of CD133 positive, negative and total Y79 cells treated with various concentrations of Carboplatin for 48 hours by MTT Assay

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