World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:11, No:02, 2017

Co-Culture with Murine Stromal Cells Enhances the In-vitro Expansion of Hematopoietic Stem Cells in Response to Low Concentrations of Trans-Resveratrol

Authors : Mariyah Poonawala, Selvan Ravindran, Anuradha Vaidya

Abstract : Despite much progress in understanding the regulatory factors and cytokines that support the maturation of the various cell lineages of the hematopoietic system, factors that govern the self-renewal and proliferation of hematopoietic stem cells (HSCs) is still a grey area of research. Hematopoietic stem cell transplantation (HSCT) has evolved over the years and gained tremendous importance in the treatment of both malignant and non-malignant diseases. However, factors such as graft rejection and multiple organ failure have challenged HSCT from time to time, underscoring the urgent need for development of milder processes for successful hematopoietic transplantation. An emerging concept in the field of stem cell biology states that the interactions between the bone-marrow micro-environment and the hematopoietic stem and progenitor cells is essential for regulation, maintenance, commitment and proliferation of stem cells. Understanding the role of mesenchymal stromal cells in modulating the functionality of HSCs is, therefore, an important area of research. Trans-resveratrol has been extensively studied for its various properties to combat and prevent cancer, diabetes and cardiovascular diseases etc. The aim of the present study was to understand the effect of trans-resveratrol on HSCs using single and co-culture systems. We have used KG1a cells since it is a well accepted hematopoietic stem cell model system. Our preliminary experiments showed that low concentrations of trans-resveratrol stimulated the HSCs to undergo proliferation whereas high concentrations of trans-resveratrol did not stimulate the cells to proliferate. We used a murine fibroblast cell line, M210B4, as a stromal feeder layer. On culturing the KG1a cells with M210B4 cells, we observed that the stimulatory as well as inhibitory effects of trans-resveratrol at low and high concentrations respectively, were enhanced. Our further experiments showed that low concentration of trans-resveratrol reduced the generation of reactive oxygen species (ROS) and nitric oxide (NO) whereas high concentrations increased the oxidative stress in KG1a cells. We speculated that perhaps the oxidative stress was imposing inhibitory effects at high concentration and the same was confirmed by performing an apoptotic assay. Furthermore, cell cycle analysis and growth kinetic experiments provided evidence that low concentration of trans-resveratrol reduced the doubling time of the cells. Our hypothesis is that perhaps at low concentration of trans-resveratrol the cells get pushed into the G0/G1 phase and re-enter the cell cycle resulting in their proliferation, whereas at high concentration the cells are perhaps arrested at G2/M phase or at cytokinesis and therefore undergo apoptosis. Liquid Chromatography-Quantitative-Time of Flight–Mass Spectroscopy (LC-Q-TOF MS) analyses indicated the presence of trans-resveratrol and its metabolite(s) in the supernatant of the co-cultured cells incubated with high concentration of trans-resveratrol. We conjecture that perhaps the metabolites of trans-resveratrol are responsible for the apoptosis observed at the high concentration. Our findings may shed light on the unsolved problems in the in vitro expansion of stem cells and may have implications in the ex vivo manipulation of HSCs for therapeutic purposes.

Keywords : co-culture system, hematopoietic micro-environment, KG1a cell line, M210B4 cell line, trans-resveratrol
Conference Title : ICSCB 2017 : International Conference on Stem Cell Biology
Conference Location : Paris, France
Conference Dates : February 23-24, 2017