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Deciphering Tumor Stroma Interactions in Retinoblastoma

Authors : Rajeswari Raguraman, Sowmya Parameswaran, Krishnakumar Subramanian, Jagat Kanwar, Rupinder Kanwar

Abstract : Background: Tumor microenvironment has been implicated in several cancers to regulate cell growth, invasion and metastasis culminating in outcome of therapy. Tumor stroma consists of multiple cell types that are in constant cross-talk with the tumor cells to favour a pro-tumorigenic environment. Not much is known about the existence of tumor microenvironment in the pediatric intraocular malignancy, Retinoblastoma (RB). In the present study, we aim to understand the multiple stromal cellular subtypes and tumor stromal interactions expressed in RB tumors. Materials and Methods: Immunohistochemistry for stromal cell markers CD31, CD68, alpha-smooth muscle (α-SMA), vimentin and glial fibrillary acidic protein (GFAP) was performed on formalin fixed paraffin embedded tissues sections of RB (n=12). The differential expression of stromal target molecules; fibroblast activation protein (FAP), tenascin-C (TNC), osteopontin (SPP1), bone marrow stromal antigen 2 (BST2), stromal derived factor 2 and 4 (SDF2 and SDF4) in primary RB tumors (n=20) and normal retina (n=5) was studied by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and Western blotting. The differential expression was correlated with the histopathological features of RB. The interaction between RB cell lines (Weri-Rb-1, NCC-RbC-51) and Bone marrow stromal cells (BMSC) was also studied using direct co-culture and indirect co-culture methods. The functional effect of the co-culture methods on the RB cells was evaluated by invasion and proliferation assays. Global gene expression was studied by using Affymetrix 3’ IVT microarray. Pathway prediction was performed using KEGG and the key molecules were validated using qRT-PCR. Results: The immunohistochemistry revealed the presence of several stromal cell types such as endothelial cells (CD31+;Vim+/-); macrophages (CD68+;Vim+/-); Fibroblasts (Vim+; CD31-;CD68-);myofibroblasts (α-SMA+/ Vim+) and invading retinal astrocytes/ differentiated retinal glia (GFAP+; Vim+). A characteristic distribution of these stromal cell types was observed in the tumor microenvironment, with endothelial cells predominantly seen in blood vessels and macrophages near actively proliferating tumor or necrotic areas. Retinal astrocytes and glia were predominant near the optic nerve regions in invasive tumors with sparse distribution in tumor foci. Fibroblasts were widely distributed with rare evidence of myofibroblasts in the tumor. Both gene and protein expression revealed statistically significant ($P<0.05$) up-regulation of FAP, TNC and BST2 in primary RB tumors compared to the normal retina. Co-culture of BMSC with RB cells promoted invasion and proliferation of RB cells in direct and indirect contact methods respectively. Direct co-culture of RB cell lines with BMSC resulted in gene expression changes in ECM-receptor interaction, focal adhesion, IL-8 and TGF-β signaling pathways associated with cancer. In contrast, various metabolic pathways such a glucose, fructose and amino acid metabolism were significantly altered under the indirect co-culture condition. Conclusion: The study suggests that the close interaction between RB cells and the stroma might be involved in RB tumor invasion and progression which is likely to be mediated by ECM-receptor interactions and secretory factors. Targeting the tumor stroma would be an attractive option for redesigning treatment strategies for RB.

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