CIRCULATING ALTRUISTIC STEM CELLS AS A MARKER OF IMMUNOSUPPRESSION IN ORAL CANCER

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Abstracts

Background Head & neck cancer is the 4th cancer, and most of them are diagnosed late, and treated with cisplatin monotherapy. In these subsets of patients, immunotherapy has been tried, but without major success.1 We speculate that circulating altruistic stem cells may serve as biomarker for immunotherapy. Previously, we showed that oral cancer CSCs reprogram MSCs to altruistic phenotype,2, 3 an embryonic stem cell like phenotype having niche defending ability.3 Here, we propose to test the immunosuppressive ability of ASCs, and their detection in the circulation of head & neck cancer subjects undergoing platinum based chemotherapy.

Methods We obtained ASCs by culturing CD271+ BM-MSCs with the conditioned media of EPCAM+/ABC2G2+ CSCs of SCC-25 cancer cell line or primary tumors as previously described. The immunosuppression test: immunosuppressive secretory factors, mixed lymphocyte reaction (MLR) assay, and a Boyden chamber based co-culture assay with NK cells.4 Next, we obtained circulating CD271+/CD45− cells of stage IV head and neck cancer subjects (n=20) (5). Kaplan-Meier survival analysis and log-rank test to find association between the presence of circulatory ASCs and overall survival (OS).

Results CSCs including primary tumor obtained CSCs reprogram CD271+ MSCs to ASC phenotype; these cells secrete high level of Nitric oxide, IDO, TGF-beta, IL-10 and PGE-2. ASCs exhibited marked immunosuppression in the MLR test. In Boyden chamber assay, ASCs markedly decreased the number of NKGD2+ NK cells, and CD8+ T cells by 5-8 fold (p<0.02; n=3), whereas increased the CD4+/FoxP3+/CD25− T-reg cells by 3-fold (p<0.05, n= 4). Moreover, ASCs, upon co-culture, increases the clonogenic capacity of non-CSCs (EPCAM+/ABC2G2−/ALDH−) cells by 5-fold (p<0.02; n=4), while significantly decreasing the secretion of NKGD2L of these cancer cells. Next, we isolated circulating CD271+/CD45− cells from the 12/20 subjects with oral cancer and confirmed their ASC phenotype (figure 1A-E). These cells were in vitro cultured (figure 1D), and the conditioned media obtained showed marked immunosuppressive activities including significant reduction of NKGD2+ NK cells in the in vitro Boyden chamber assay. Importantly, these 12 out of 20 patients showed poor treatment response to platinum therapy. The OS at 6 months was 24.6% for circulating ASC-positive patients and 47.4% for circulating ASC-negative patients (log-rank test, p<0.001).

Conclusions The circulatory ASCs could be a promising biomarkers for immunotherapy.

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Ethics Approval The clinical study was approved by ‘Institutional Ethics Committee’ of KaviKrishna Laboratory, Guwahati, India and KaviKrishna Telemedicine Care, Sualkuchi, Assam, India. The stem cell study was conducted following the guidelines of NAC-SCRT and was approved by Institutional Committee for Stem Cell Research (ICSCR), KaviKrishna.

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A. Representative flow cytometry profile depicting the presence of CD271+ cells in a population of EpCam-/CD45− cells sorted from peripheral blood mononuclear cells (PB-MNCs) of subjects with Oral cancer (supplementary table 4). The PB-MNCs were subjected to immunomagnetic sorting for EpCam-/CD45+ cells. The sorted population was then mixed with CD271+/CD45+ cells recovered from the same subject for 1-day in serum free medium, fixed in 4% formaldehyde, and subjected to CD271 staining. The mixing with CD271+/CD45+ cell was done to minimize cell loss during flow cytometry procedure. B. The quantitative data of viable circulating CD271+/CD45− cells was estimated by trypan blue exclusion assay. Healthy subjects did not exhibit circulating CD271+/CD45− cells. C. Relative CFU-F was estimated in the isolated circulating CD271+/CD45− cells to confirm the mesenchymal phenotype. High Grade Oral Cancer (HG cancer) CFUs: 4.2 ±1.5/103cells (n=4). D. Relative protein level of p53 as measured by In-cell ELISA of CD271+/CD45− cells cultured in vitro in serum free medium. E. Gene expression profiles of circulating CD271+/CD45− cells. Note that circulating CD271+ cells exhibited enhanced expression of HIF-2α, Nanog, Sox-2 and Oct-4. The CD271+/CD45− cells were cultured in the serum free StemSpan medium for one week to obtain mRNA. The values were compared with Day-8 CM treated MScs. F. CM of circulating CD271+/CD45− cells obtained from HG cancer exhibited higher capacity to enhance the tumorigenicity of non-SP cells compared to CM of R-Mscs (Day-8, reprogrammed by SCC-25 ABCG2+SC-CM).