Identification of myeloid-derived suppressor cells in squamous cell cancer of the head and neck

Grace G. Kim, MD1,2; Adam M. Zanation, MD1; Carol G. Shores, MD, PhD1; Karen P. McKinnon, PhD2; Dominic T. Moore, MPH2; Jonathan S. Serody, MD2,3

1Department of Otolaryngology – Head & Neck Surgery, University of North Carolina – Chapel Hill, 2Departments of Medicine, Microbiology and Immunology 3Lineberger Cancer Center, University of North Carolina – Chapel Hill

INTRODUCTION

- Squamous cell carcinoma of the head and neck (SCCHN) is known to be immunosuppressive and myeloid-derived suppressor cells (MDSCs) may play an important role in tumor progression in patients with head and neck cancer.
- MDSCs are a heterogeneous population of immature myeloid cells including precursors to granulocytes, monocytes, macrophages, and dendritic cells [1, 2].
- MDSCs directly inhibit T cell function by production of arginase I and II [3], nitric oxide [4], and reactive oxygen species [5].
- MDSCs also have indirect contributions to tumorigenesis by inducing regulatory T cells [6] and T helper immune response [7], suppression of natural killer cells [8], and increasing angiogenesis [9].
- The objective of this feasibility study is to report the first large cohort identification of MDSCs in patients with SCCHN.

METHODS AND MATERIALS

- Patients with SCCHN who underwent tumor resection between August 2011 to July 2012 (Table 1).
- PBMCs were isolated from venous blood by density gradient sedimentation using Ficoll-Hypaque.
- Tumor-infiltrating leukocytes were isolated by mechanically and chemically digesting solid tumor with collagenase type IV, hyaluronidase, and protease inhibitors.
- Monoclonal antibodies were used for surface staining of MDSCs: CD45+, CD34+, CD14+, and CD11b+.
- Multiple parameters flow cytometry for MDSCs was performed using the MACSQuant Analyzer (MiltenyiBiotec, Auburn, CA, USA).

RESULTS

- We selected for MDSCs by characterizing cells that expressed the common leukocyte antigen (CD45+).
- MDSCs were reliably identified as CD33+CD11b+CD14- cells.
- A representative patient with squamous cell carcinoma of the paranasal sinuses demonstrates the gating strategy used for peripheral blood (Figure 1) and tumor (Figure 2).
- The frequency of MDSCs in peripheral blood was 3.6% (95% CI=1.23%) (n=26).
- In contrast, the frequency of MDSCs at the tumor site was 14.7% (95% CI=4.4-22.8%) of total leukocytes (n=8) of patients with SCCHN.

DISCUSSION

- There is limited information on frequencies of MDSCs in large cohorts of patients with SCCHN.
- The heterogeneity of MDSCs makes identifying MDSCs in cancer patients challenging and little is known about the antitumor response of MDSCs in head and neck cancer.
- Isolation of MDSCs from tumor specimens of the head and neck is yet to be defined in sufficient numbers and compared to MDSCs in the peripheral blood.
- Early protocols performed by our laboratory to identify MDSCs from isolated cells of tumors that have been incubated at 37°C for 1-4 days proved to be very difficult, likely due to activation and differentiation of MDSCs mediated by cytokines produced in the tissue culture environment.
- To prevent maturation, reliable assessments of MDSC frequencies were performed on fresh blood and tumor samples using low cluster tissue culture plates.
- Our study demonstrates higher MDSC frequencies in tumor infiltrating leukocytes than peripheral blood, suggesting that there is a preferential accumulation of MDSCs in the tumor site.

CONCLUSIONS

- This study demonstrates feasibility of identifying higher percentage of MDSCs in tumors compared to blood in a large cohort of patients with head and neck cancer.
- Characterization of MDSCs in SCCHN can have implications for therapeutic strategies including chemotherapeutic agents and immunotherapeutic strategies.
- Further studies on functional roles of MDSCs in head and neck cancer are warranted.

REFERENCES