Comparative Analysis of Tumor Infiltrating Lymphocytes in a New Syngeneic Model of Oral Cancer

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Abstract

Objective: To perform a comparative analysis of infiltrating immune cells in a newly developed C57BL/6 background syngeneic transplantable mouse oral cancer (MOC) model.

Methods: Use of tissue culture, cell line transplantation, and flow cytometric analysis.

Results: Previously, we established a series of cell line models that displayed dichotomous growth phenotypes when transplanted into immunocompetent mice. Now we show that the indolent growth pattern of the MOC1 generated tumors is associated with increased baseline and inducible MHC class I expression and increased CD8+ T-cell infiltration into the tumor microenvironment. Conversely, the aggressive and metastatic pattern of MOC2 generated tumors has decreased basal and inducible class I expression and is associated with FOXP3+/CD4+ regulatory T-cell infiltration. Delayed primary tumor growth after targeted monoclonal antibody therapy of these FOXP3+ regulatory T-cells further suggests that these immune cells contribute to the aggressive phenotype of MOC2-generated tumors.

Conclusions: These data validate that key infiltrating immune cells identified here parallel findings in human head and neck cancer, making this newly developed syngeneic model a critical platform for the continued dissection of tumor-host interactions in head and neck cancer.

Methods

Animals: C57BL/6 wildtype and Rag2−/− mice were purchased from Taconic. Animal Studies and Research Ethics Committees of Washington University in St. Louis approved all animal studies.

Cell lines: Syngeneic C57BL/6 mouse oral cancer (MOC) cell lines were generated as described previously. Limited understanding of host immune responses in HNSCC stems from the void of immunocompetent pre-clinical models. To address this paucity, our laboratory has created a transplantable C57BL/6 syngeneic model of HNSCC, which we have previously classified cell lines as either aggressive or indolent phenotypes.

RESULTS

Figure 1: Growth rates and class I expression of MOC lines. A. Growth curves of transplanted MOC lines. B. Class I expression at baseline and after IFN-γ stimulation. C. Baseline expression of KIR on MOI lines. D. Induced expression of KIR on MOI lines.

Figure 2: Expanded CD11b+Gr1+ cells in MOC lines. A. Representative FACS data from mouse spleens. B. Representative data from MOC generated tumors. C. FACS data from regional lymph nodes. H&E stained sections of tumor-draining lymph nodes.

Figure 3: CD11b+Gr1+ cells are F4/80+. A. Representative FACS plots of MOC1 or MOC2 generated tumor microenvironments. CD11b+Gr1+ cells represent an F4/80-negative control.

Figure 4: T-cell infiltration in the tumor microenvironment. A. CD4+ and CD8+ cells in MOC generated tumors. B. % live CD45+ CD8+ cells in the tumor microenvironment (%p<0.05). C. % live CD45+ CD8+ in the tumor microenvironment (%p<0.001).

Figure 5: Depletion with anti-CD25 (PC61) attenuates MOC2 primary tumor growth. A. CD4+ T-cells in the tumor microenvironement. B. FoxP3 in the tumor microenviroment. C. MOC2 tumors treated with HRPN or PC61. (%p<0.05)

Conclusions

In summary, we have described the first comparative infiltrating immune cell analysis of two cell lines that display either aggressive or indolent growth phenotypes. We identified increased major histocompatibility complex (MHC) class I expression and CD8+ T-cell infiltration in tumors generated from the less aggressive cell line. Additionally, we found that a significant portion of CD4+ T-cells infiltrating tumors generated from the more aggressive cell line are immunosuppressive FOXP3+ regulatory T-cells, and that antibody-mediated inhibition of these Tregs leads to attenuated growth. Primary tumors generated from both cell lines, as well as secondary lymph nodes, demonstrated similar metastatic disease. Primary tumors generated from the more aggressive cell line, demonstrate robust infiltration of CD11b+Gr1+ cells. These data recapitulate the infiltrating lymphocyte profile observed in human HNSCCs and validate the utility of using this syngeneic oral cancer model to study tumor-host interactions within the tumor and metastatic microenvironment.

References
