Correlation of Regulatory T cell Prevalence and Function in Head and Neck Melanoma Sentinel Lymph Nodes to Clinical Outcome

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ABSTRACT

Objective: Sentinel lymph node status is an important prognostic factor in head and neck melanoma. Regulatory T cells expressing FoxP3 suppress tumor immunity and indoleamine 2,3-dioxygenase is an enzyme that promotes regulatory T cell activation. Research objectives were: 1) Assess FoxP3/indoleamine 2,3-dioxygenase immunoreactivity in head and neck melanoma sentinel lymph nodes and 2) Correlate FoxP3/indoleamine 2,3-dioxygenase with sentinel lymph node metastasis and clinical outcome.

Study Design: Retrospective cohort study with immunohistochemical correlation

Setting: Tertiary medical center.

Subjects and Methods: Analysis of patients with sentinel lymph node biopsy for head and neck melanoma between 3/2004 and 9/2011. FoxP3/indoleamine 2,3-dioxygenase prevalence and intensity were determined from immunohistochemistry of the nodes. An overall immunoreactivity score was correlated with outcome and sentinel lymph node metastasis using the Chi-square test for trend.

Results: 56 sentinel lymph nodes were reviewed, with 47 negative and 9 positive for melanoma. Patients with poor outcomes had a statistically significant trend for higher scores (p=0.03). Positive compared to negative nodes also had a statistically significant trend for higher scores (p=0.03). Amongst the negative nodes, there was a statistically significant trend for a poor outcome with higher score (p=0.02).

Conclusion: FoxP3/indoleamine 2,3-dioxygenase immunoreactivity correlates with sentinel lymph node positivity and poor outcome. Even in negative nodes, higher immunoreactivity correlated with poor outcome. Therefore higher immunoreactivity may portend a worse prognosis even without regional metastasis at the time of sentinel lymph node biopsy. This subset of patients may need closer follow-up and more adjuvant treatment than previously recommended.

INTRODUCTION

Melanoma incidence is the fastest rising of any malignancy [1,2]. 18% of cases are in the head and neck (H&N) and these have a worse prognosis [3,4]. Overall, the most important prognostic factor is the number of nodal metastases [5]. In patients with clinically negative lymph nodes (LNs), the sentinel lymph node (SLN) status is the most important prognostic factor [6].

SLN biopsy has replaced elective lymph node dissection as the standard to determine subclinical LN metastases. A +SLN in the H&N leads to a completion neck dissection (CND) while a -SLN avoids this morbidity. Despite SLN biopsy, the death rate from melanoma continued to rise from 1990-2006 [7].

Immunosuppressive regulatory T cells (Tregs) in LNs and allow tumor immune escape. When activated, they express the transcription repressor FoxP3. Indoleamine 2,3-dioxygenase (IDO) is an enzyme that promotes Treg activation. Increased Tregs have been associated with a variety of tumors, including melanoma [8,9].

METHODS AND MATERIALS

• Patients in the Duke Tumor Registry with a new diagnosis of H&N melanoma who underwent SLN biopsy from 2004-2011
• Poor outcome was local, regional or distant recurrence
• Immunohistochemistry for FoxP3 and IDO on SLN was scored according to Table 1 then combined for a total FoxP3/IDO score
• Chi-Square test for trend compared immunoreactivity, SLN metastases and outcome

RESULTS

• 56 SLNs from 30 patients. 47 SLNs had good and 9 poor outcome. 47 -SLNs and 9 +SLNs. Characteristics in Table 2. 19 SLNs had low, 26 intermediate and 11 high scores.
• SLNs with a poor outcome had a trend towards higher scores 11% (n=1) low, 44% (4) intermediate and 44% (4) high vs. a good outcome with 38% (18) low, 47% (22) intermediate and 15% (7) high (p=0.03). Figure 1A.
• +SLNs had a trend towards higher scores with 22% (2) low, 22% (2) intermediate and 56% (5) high vs. -SLNs with 36% (17) low, 51% (24) intermediate and 13% (6) high (p=0.03). Figure 1B.
• -SLNs with a poor outcome had a trend toward higher scores with 0% (0) low, 60% (3) intermediate and 40% (2) high vs. +SLNs with good outcome with 40% (17) low, 50% (21) intermediate and 10% (4) high (p=0.02). Figure 1C.

DISCUSSION

We found a trend towards higher FoxP3/IDO scores in +SLNs vs. -SLNs and in poor vs. good clinical outcome. Both of these findings support previous studies [10,11].

Most interestingly, the FoxP3/IDO score correlated with poor outcome even with -SLNs, which was also recently shown in all-site melanoma [12]. With -SLN a CND, adjuvant immunotherapy may allow Treg inhibition in clinical outcome. A significant role in clinical outcome of activated Tregs even in -SLN and high FoxP3/IDO may suggest the need for more aggressive treatment and follow-up.

There are no previous publications comparing Treg and IDO expression with outcome in H&N melanoma. Given the complex and sometimes unexpected SLN drainage pathways in the H&N, as well as generally worse outcomes, consideration of this specific subpopulation is important.

Melanoma is resistant to most chemotherapy. Ipilimumab is a monoclonal antibody that blocks CTLA-4, to downregulate Tregs. It shows an improvement in overall survival in metastatic melanoma, but many patients derive no benefit from it [13]. IDO inhibition may allow Treg inhibition in those cases [14]. Agents that block both Tregs and IDO are being studied in the basic science, translational and clinical settings.

CONCLUSIONS

• The addition of FoxP3/IDO immunohistochemistry to melanoma SLN protocols may capture a broader population of patients that need more aggressive management.
• Future directions include a blinded prospective study validating the correlation between FoxP3/IDO immunoreactivity and outcome in this study.
• Treg and IDO analysis on SLN could be an inclusion criterion for future clinical trials investigating new therapies targeting Tregs, IDO and immune system tumor escape.

REFERENCES


Table 1. Modified German Immunoreactive Scoring System based on stain intensity and prevalence of positive cells

Table 2. Patient and tumor characteristics

Figure 1. Percentage of each IR score by (A) clinical outcome (B) SLN tumor metastasis status and (C) clinical outcome for all –SLNs. These show a statistically significant trend towards higher FoxP3/IDO immunoreactivity with +SLN and with poor outcome in both +SLN and –SLN.

Figure 2. Photomicrographs at 40X of –SLN with low (A) FoxP3 and (B) IDO immunoreactivity in a patient with a good outcome. Compared to +SLN with high (C) FoxP3 and (D) IDO in a patient with a poor outcome.

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