ORIGINAL ARTICLE

The distribution of cutaneous metastases correlates with local immunologic milieu

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Background: Metastases to the skin are found with increased frequency at certain sites, such as the scalp, but the biological factors that influence this distribution are not understood.

Objective: We aimed to compare the proportional frequency of metastases at various cutaneous locations with the immunologic microenvironments at those sites.

Methods: We retrospectively identified all biopsy specimens of cutaneous metastases diagnosed at our institution from 1991 to 2014 (n = 1984) and mapped their anatomic distribution while controlling for regional surface area. Using a separate, mapped cohort of normal-appearing skin samples (n = 140), we measured the density of regulatory T cells, $CD4^+$ effector T cells, and $CD8^+$ T cells by flow cytometry.

Results: Per unit surface area, cutaneous metastases arise most commonly on the head and neck, followed by the trunk, upper extremities, and lower extremities, respectively. Sites with more frequent metastases tend to contain a greater density of regulatory T cells and a lower proportion of $CD8^+$ T cells ($P < .05$).

Limitations: Immunologic factors were only assessed in control tissue and were not measured from patients with metastatic disease in this correlative single-center study.

Conclusion: The distribution of cutaneous metastases follows the distribution of regulatory and effector T cells in skin. Further studies are required to prove a mechanistic association between local immunologic factors and the development of cutaneous metastases. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2015.10.012.)

Key words: anatomic distribution; cutaneous metastasis; effector T cells; immunology; metastatic cancer; regulatory T cells.

The skin is an organ commonly affected by metastatic cancer. Approximately 10% of all metastases are cutaneous, and roughly 1% to 2% of all internal malignancies will eventually metastasize to the skin.[1,2] For some types of metastatic cancer, cutaneous spread is particularly frequent: about half of all melanoma metastases and roughly one third of breast cancer metastases involve the skin.[1] Accordingly, dermatologists and dermatopathologists are often in a position to diagnose and monitor metastatic disease.

Despite the frequency with which metastases involve the skin, little is known about the biologic mechanisms that underlie their development. Early studies of cutaneous metastasis focused mostly on epidemiology, primary tumor types, and the anatomic regions of the skin most commonly involved.[1,3] Among men, lung cancer, colon cancer,

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and melanoma have been the tumors that most frequently metastasize to skin; whereas for women, the overwhelming majority of metastases are of breast origin.[3,4] In both genders, the trunk has been identified as a site of predilection, with the head and neck region commonly involved as well.[1,4] However, these data have not been controlled for regional surface area, thereby limiting comparison among sites.

Recent advances in immunology have highlighted the importance of immune surveillance in tumor development and progression. In this study, we seek to integrate local immunologic factors into a model of cutaneous metastasis. Using the largest—to our knowledge—cohort to date, we map, in controlled fashion, the distribution of cutaneous metastases. By correlating these maps with tissue-specific immunologic data, we propose that the immunologic milieu of the skin plays a critical role in the distribution of cutaneous metastasis.

CAPSULE SUMMARY

- Cutaneous metastases are distributed in nonrandom fashion.
- The distribution of cutaneous metastases can be predicted by variations in T-cell populations within the skin.
- Interventions that modulate the immune system hold the potential to influence metastatic spread.

METHODS

Metastasis data collection and mapping

The archival database of the University of California, San Francisco, Dermatopathology Service was searched for all cases of cutaneous metastasis from solid-organ tumors diagnosed between January 1, 1991, and November 17, 2014, after institutional review board approval. All diagnoses were made by a board-certified dermatopathologist and supported by a combination of clinical, histopathologic, and immunohistochemical features indicative of metastasis. Exclusion criteria were noncutaneous biopsy site, unspecified cutaneous biopsy site, and inconclusive evidence of metastasis. When multiple biopsy specimens were obtained from the same patient on the same day from the same anatomic site, those biopsy specimens were counted as a single metastasis. For each case, the anatomic location of the metastasis and the tumor type from which it originated, if known, were recorded. All data were recorded and analyzed in an anonymized fashion.

Proportional frequency of metastasis at a given anatomic site was calculated by dividing the number of metastases at that site by the total number of all metastases, and then dividing again by the percent body surface area occupied by that anatomic site. Percent body surface area was estimated using the "rule of nines."[5] Heat maps were created by assigning a color to each anatomic region based on the region's proportional frequency of metastasis, using software (Excel 2010, Microsoft Corp, Redmond, WA) to arbitrarily assign red to the highest proportional frequency, yellow to the median, and green to the lowest, with intermediate colors determined proportionately.

Flow cytometric immunophenotyping of normal-appearing skin

Immune cell subsets were quantified from normal-appearing skin using flow cytometry as previously described.[6] Briefly, normal-appearing human skin was obtained from patients at University of California, San Francisco, undergoing elective surgery, in which healthy skin was discarded as a routine procedure. Subcutaneous fat and hair were removed, and skin was minced finely with dissection scissors and mixed in a 6-well plate with 3 mL of digestion buffer consisting of 0.8 mg/mL collagenase type 4 (4188, Worthington, Lakewood, NJ), 0.02 mg/mL DNAse (DN25-1G, Sigma-Aldrich, St Louis, MO), 10% FBS, 1% Hepes, and 1% penicillin/streptavidin in RPMI medium. Samples were incubated overnight in 5% CO2, harvested with wash buffer (2% FBS, 1% penicillin/streptavidin in RPMI medium), and filtered through a 100-μm filter, centrifuged, counted, and stained for flow cytometry. The following antibodies were used for flow cytometry: anti-hCD3 (-FITC, eBioscience, San Diego, CA), anti-hCD4 (-PerCP-eFluor710, eBioscience), anti-CD8 (-BV605, BD Biosciences, San Jose, CA), anti-hFoxP3 (-eFluor 450, eBioscience), and LIVE/DEAD Fixable Aqua Dead Cell Stain (Life Technologies, South San Francisco, CA). Data were acquired by an LSRFortessa (BD Biosciences) and analyzed using FlowJo software (Tree Star Inc, Ashland, OR).

Statistical analysis

Immunophenotyping data followed a gaussian distribution and variation was similar between groups for each group. Significance was assessed using the unpaired Student *t* test. In all dot plots, the mean value ± SEM is visually depicted. Statistical analysis was done using GraphPad Prism software (GraphPad, San Diego, CA).