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Lymphatics, Lymph Nodes and the Immune System: Barriers and Gateways for Cancer Spread

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Metastasis to the regional lymph node is the most important prognostic indicator for the outcomes of patients with sold cancer. In general, it is well recognized that cancer development is genetically determined with progression from the microenvironment of the primary tumor site, oftentimes via the SLN gateway, to the distant sites. In about 20% of the time, the cancer cells may spread directly through the blood vascular system to the distant sites. Thus, in general, cancer progression is consistent with Hellman's spectrum theory in that development of nodal and systemic metastasis from a localized cancer growth is a progressive process. Cancer proliferation within the tumor microenvironment may give rise to increased tumor heterogeneity, which is further complicated by its continuous change through its evolution within the host in a Darwinian sense. It is crucial to understand the molecular process of lymphangiogenesis and hemangiogenesis in the tumor microenvironment with respect to the initial steps of cancer cells entering into the lymphatic and vascular systems so that rational therapy can be developed to curb the process of specific routes of metastasis. This chapter elucidates the role of lymphatics, nodal metastasis and antitumor immunity. We present novel immune targets in nodal metastases, the importance of the lymph node as a pre-metastatic niche, and immune-related proteins

as biomarkers of metastasis

lymphatic metastasis; premetastatic niche; antitumor immunity; immune biomarker

Introduction

Sentinel Lymph Node as the Gateway to Cancer Spread

Stanley P. L. Leong, MD, FACS

Metastasis to the regional lymph node is the most important prognostic indicator for the outcomes of patients with sold cancer. In general, the fact that cancer cells proliferate within the tumor microenvironment and spread to the regional sentinel lymph node(s) first, then beyond to the systemic sites, has been well established in melanoma (1) and breast cancer (2). This concept is further being validated in other solid cancers including cancers of the head and neck, gastrointestinal tract, genitourinary tract, gynecological sites and the lung (3). The “seed and soil” theory of Paget ((4)) emphasizes that the interaction of spreading cells and the microenvironment of the metastatic niche is non-random. In general, it is well recognized that cancer development is genetically determined with progression from the microenvironment of the primary tumor site, oftentimes via the SLN gateway, to the distant sites. In about 20% of the time, the cancer cells may spread directly through the blood vascular system to the distant sites ((3)). Thus, in general, cancer progression is consistent with Hellman’s spectrum theory in that development of nodal and systemic metastasis from a localized cancer growth is a progressive process ((5)). Cancer proliferation within the tumor microenvironment may give rise to increased tumor heterogeneity, which is further complicated by its continuous change through its evolution within the host in a Darvinian sense ((6)). It is crucial to understand the molecular process of lymphangiogenesis and

hemangiogenesis in the tumor microenvironment with respect to the initial steps of cancer cells entering into the lymphatic and vascular systems (7–11) so that rational therapy can be developed to curb the process of specific routes of metastasis (12–14). In each step of progression, specific biomarkers may be independent prognosticators for melanoma, e.g. thickness and ulceration of melanoma are specific and independent prognostic factors from the nodal status. Recently, the mitotic rate of the primary melanoma has been shown to have prognostic significance ((15)) and it has been incorporated in melanoma stating the 7th edition of TNM ((16)). The relationship between the biology of the primary tumor and the traditional TNM stating becomes an important one as more primary tumor features are found to be of prognostic value with respect to the lymph node status or clinical outcomes. Similarly in breast cancer, the number of positive nodes is of prognostic significance. The inclusion of SLN status as a prognostic biomarker in the 7th edition of the AJCC Caner Staging Manual (http://www.cancerstaging.net) for breast cancer and melanoma is very appropriate. Thus, the suffix (sn) has been used to designate a sentinel node to be positive and negative only in contrast to a node from a complete axillary or regional dissection. Melanoma is an excellent model to study cancer metastasis from proliferation within the tumor microenvironment, spreading to the regional SLNs in an orderly fashion and beyond to the distant sites ((3)). In the sentinel lymph node era, the spectrum of lymph node involvement ranges from isolated tumor cells (ITCs, a cell or a cluster of cells less than 0.2 mm) ((17, 18)), micrometastasis (tumor less than 2 mm), macrometastasis (over 2 mm) and palpable nodes. Our recent work and others on melanoma SLN micrometastasis shows that different amount of tumor burden in SLN is correlated with various clinical outcomes of patients with less tumor burden being associated with more favorable outcome. Further, we have found that both primary melanoma thickness and SLN tumor burden progressively affect the disease-free survival and overall in melanoma patients ((19)). Thus, SLN tumor burden further supports the spectrum theory of cancer progression ((5)).

To date, the SLN concept that cancer of a specific anatomical site drains to its regional SLN(s) has been extensively validated in melanoma and breast cancer. In practice, if the SLNs are negative, a more morbid regional lymph node dissection can be avoided. In penile carcinoma, SLN mapping will guide the specific side of involvement, thus, avoiding a morbid bilateral radical ilioinguinal lymph node dissection. For other solid cancers including head and neck, colorectal cancer, upper GI cancers, other GU cancers and gynecological cancers, the anatomy is more complicated with more extensive lymphatic pathways and, thus, the lymphatic system is more unpredictable.

More studies are needed to validate the drainage patterns of lymphatic spread of these types of cancer. The goal in head and neck as well as gynecological cancers is to develop an accurate SLN mapping method to minimize the extent of lymph node dissection. While the extent of lymphadenectomy in lung, colorectal and upper GI cancers may not be altered significantly, harvesting the SLNs will increase the staging accuracy of the nodal basins, especially in view of the fact that the number of lymph nodes being removed during resection of colorectal cancer ((20)) and gastroesophageal cancer ((21)) is a significant predictor of survival. Thus, it is logical that harvesting SLNs in these cases would result in more accurate assessment of nodal status in these cancers. In summary, the concept of SLNs in solid cancers has given rise to new paradigms of cancer metastasis in that the SLNs are the important gateways for the cancer cells to spread ((3)).

Identification of Tumor Antigen Genes for Immune Targeting of Metastasis using Polymerase Chain Reaction (PCR)

Robert L. Ferris

Metastasis to cervical lymph nodes (LN) occurs in approximately 30% of patients with early squamous cell carcinoma of the head and neck (SCCHN) and is associated with regional recurrence and poor outcome ((22, 23)). Physical examination, imaging, and histopathologic characteristics of the primary tumor are not accurate enough to determine TNM stage and to guide treatment reliably. Although close observation (i.e., watchful waiting) and elective neck irradiation remain options, most clinicians favor excision of the regional lymphatics at the time of resection of the primary cancer for accurate staging. However, 60–70% of the operated patients ultimately do not have nodal disease pathologically (pN0) and therefore are theoretically over-treated ((24)). More, targeted, and less invasive approaches would limit elective neck dissection (END) in many of the patients without apparent metastatic disease. Sentinel node biopsy (SNB) allows the surgeon to identify and excise targeted “first echelon” lymph nodes that reliably drain the site of a primary malignancy ((25, 26)).

There is a need for a more sensitive method for detection of micrometastases to permit metastasis-specific immunotherapy. A promising method for SLN examination is molecular analysis. It is more sensitive and less expensive than immunohistochemistry. This method has the advantage of higher sensitivity and the possibility of examining not only a single section but also an entire lymph node in a single examination. Using quantitative reverse transcription-polymerase chain reaction (QRT-PCR), it is possible to detect 1 cancer cell in a background of 10 million lymphoid cells, far surpassing the sensitivity of other methods. RT-PCR is capable of detecting the mRNA for many tumor markers in a variety of tissues, including lymph nodes. However, the optimal markers for molecular analysis will be important to determine the sensitivity and specificity for intraoperative detection of metastasis.

Rapid molecular detection of metastasis was not possible until 2002, with description of a novel method for multiplex PCR with internal controls for performance in rapid quantitative RT-PCR assays ((27, 28)). The ability to complete PCR assay within 30 minutes makes it possible to perform intraoperative testing of lymph nodes. Hughes et al. ((29)) used rapid, multiplex QRT-PCR assay to evaluate SLNs from breast cancer patients. A validation set of 90 SLNs from breast cancer patients was prospectively characterized using 4 markers individually or in combinations, and the results compared with histologic analysis. A 2 marker assay was found to be 97.8% accurate (94% sensitivity, 100% specific) compared with histologic analysis. The fully automated GeneXpert instrument produced comparable and reproducible results in less than 35 minutes. This assay surpassed the accuracy of current frozen section analysis of SLNB specimens and is potentially superior to complete histologic and IHC analysis because of the assay is fully automated, reducing the potential for human error, objective criteria are used, removing subjective analysis and improving standardization, and the time from tissue acquisition to result is less than 35 minutes, facilitating intraoperative use ((30, 31)).

Tsujimoto et al. developed an one-step nucleic acid amplification (OSNA) assay, which consist of solubilization of a lymph node followed by reverse- transcription loop- mediated isothermal amplification (RT-LAMP) of target mRNA. They reported an efficient intraoperative detection method for lymph node metastasis in breast cancer patients using the OSNA assay with cytokeratin 19 (CK 19) mRNA as a target marker. The OSNA assay can discriminate macrometastasis from micrometastasis and micrometastasis from nonmetastasis when the cutoff values of CK 19 mRNA is properly set. The examination time was shortened within 30 minutes.

We investigated the MAGE-3 gene as an antigenic target due to overexpression in our metastatic lymph node studies ((30)). The MAGE antigens are frequently expressed cancer vaccine targets. Using QRT-PCR we evaluated the expression of MAGE-3/6 in 65 cancers

and tumor matched sites. Expression results were confirmed using Western blot. HLA-A*0201:MAGE-3-(271–279) specific cytotoxic T lymphocytes (MAGE-CTL) from SCCHN patients and healthy donors showed that MAGE-3/6 expression was highly associated with CTL recognition in vitro. On the basis of the MAGE-3/6 expression, we could identify 31 (47%) of the 65 UADT tumors, which appeared to express MAGE-3/6 at levels that correlated with efficient CTL recognition. To confirm that the level of MAGE-3 expression was responsible for CTL recognition, 2 MAGE-3/6 mRNA(high) SCCHN cell lines were subjected to MAGE-3/6-specific knockdown, showing that MAGE expression and MAGE-CTL recognition were significantly reduced. Thus, using QRT-PCR UADT cancers frequently express MAGE-3/6 at levels sufficient for CTL recognition, supporting the use of a QRT-PCR-based assay for the selection of candidates likely to respond to MAGE-3/6 immunotherapy ((32)). Other antigens have been developed in similar fashion, including p53 and EGFR ((33, 34)).

In conclusion, we have shown that QRT-PCR can detect, with high sensitivity and specificity, metastatic disease in lymph nodes of patients with SCCHN. In addition, we have showed the feasibility of automated, intraoperative staging of cervical lymph nodes and the possibility that such an approach may eventually prove superior to conventional pathology. Staging of the cN0 neck is currently a topic of intense interest in the head and neck oncologic community, with the goal that therapeutic surgical and adjuvant treatment be administered to those most likely to benefit from it. Whereas the ACOSOG Z0360 trial validated the multiple single-institution studies that suggested the utility of SLN mapping for staging the cN0 neck in SCCHN, but it is unlikely that SLN biopsy will be widely accepted without a rapid, accurate, and standardized method of staging the SLN(s). Our development of such an assay and identification of discriminatory marker genes provides the pilot data necessary for the incorporation of QRT-PCR into future clinical studies applying SLN mapping to clinical practice for patients with this disease.

B7-H3 Ligand Expression on Tumor Cells As A Potential Regulator of Metastasis

Dave S.B. Hoon

Recent studies have shown the B7 ligand family of proteins has immunoregulatory activities by human immune cells as well as tumor cells ((35–38)). The major B7 ligands include B7-H1(CD80), B7-H2(CD86), B7-H3(CD276), and B7-H4 ((38, 39)). The most studied molecules are B7-H1 and H2 due to their influential immunoregulatory activity during host immune responses such tumor, auto, and infectious disease immunity ((36, 37, 40, 41)). More recently these molecules have drawn attention due to the approval of anti-CTLA4 receptor monoclonal antibody(iplimumab) FDA approval in stage IV melanoma patients. Immunoregulatory molecules have been found to be expressed by tumor cells and suggested to have a functional role in tumor progression ((42)). We will focus on the B7-H3 ligand which has immunoregulatory properties on immune cells such as dendritic cells, monocytes/macrophages, and activated T-cells ((35–38)). This immune cell surface protein is also expressed by various tumor cells such as breast cancer, melanoma, renal cell carcinoma, lung cancer, prostate cancer, and neuroblastoma ((43–47)). The functional role of B7-H3 expression on tumor cells still remains uncertain. Studies have suggested it functions as an immunoregulatory molecule while others suggest its role is more functional in regards to tumor progression Nevertheless, this ligand is well expressed on multiple tumor types of different embryonic lineage. This molecule has wide spread diversity of expression among different cell lineages of normal and tumor cells suggesting some important functional role(s) being preserved through evolution. This is an example of an immunoregulatory

molecule well used by immune cells that is also on tumor cells. However, the role of B7-H3 on human tumor cells is not well understood.

Unfortunately the B7-H3 receptor in human cells is still unknown. This has made the studying of the functional aspects of B7-H3 somewhat difficult. Interestingly B7-H3 appears to be more abundantly expressed on human tumor cells than the other B7 family members. B7-H1 and H2 in general are expressed at very low levels on tumor cells or not at all. B7-H3, is a type I transmembrane domain, having two isoforms of Ig family members containing two domains ((47)). The actual structural is still not fully known and controversies have occurred over the years. Further studies are needed in cloning and sequencing the B7-H3 complex. Recently, we have demonstrated the expression of B7-H3 on primary and metastatic breast cancer cells ((47)). We demonstrated B7-H3 expression by quantitative RT-PCR, immunohistochemistry(IHC), and immunocytochemistry and flow cytometry. B7-H3 expression by primary tumors was approximately 39%. The study demonstrated B7-H3 expression by primary tumor is significantly correlated to increasing tumor burden, AJCC stage, and lymphovascular invasion ((47)). Using a multivariate analysis B7-H3 expression by primary tumors significantly predicted regional lymph node metastasis. The level of B7-H3 expression of primary tumor significantly correlated to the number of metastatic lymph nodes. In an analysis of primary tumor and sentinel lymph node (SLN) when the SLN was detected it was positive for B7-H3 ((47)). Distant organ metastasis was also shown to be positive for B7-H3 expression. These studies demonstrated for the first time the significant role of B7-H3 in early primary tumor progression and metastasis. Currently, we are evaluating B7-H3 expression as a prognostic factor in primary breast cancer patients. The studies suggest that B7-H3 may be a significant factor in regional lymph node metastasis establishment. The functional role in this situation is not understood. It could be due to immunoregulatory modulation or activation of specific tumor cell properties. In recent studies (to be published) B7-H3 expression in melanoma cells has been shown to induce factors promoting metastasis.

Our studies and others suggest that B7-H3 expression on tumor cells has an important clinical and pathological role. Further studies are needed in the mechanistic role of B7-H3 as related to metastasis. Future studies on developing targeted therapy to B7-H3 may be of clinical value in controlling high expressing B7-H3 tumors. The findings of B7-H3 further support how immune regulatory molecules are expressed on tumor cells and likely to influence tumor progression. This study was supported by the Leslie and Susan Gonda (Goldschmied) Foundation (Los Angeles) and the Associates for Breast and Prostate Cancer Studies (Los Angeles).

Bad Apples in a Good Neighborhood: The Lymph Node as a Premetastatic Niche for Cancer

Michael Lotze

Hanahan and Weinberg presented their six original hallmarks of cancer ((48)) with a recent updateadding two emerging hallmarks ((49)). One is avoiding immune destruction, and a secondary characteristic of arising in the setting of tumor-promoting inflammation. Deregulatted cellular energetics, (the Warburg Hypothesis) of the cancer's cells shift in metabolism to primarily glycolysis and away from oxidative phosphorylation. This may be mediated by fundamental differences in the autophagic clearance of mitochondria (mitophagy), necessary for rapidly proliferating cells and remodeling tissues. A critical nuclear protein, high mobility group box 1 (HMGB1) appears to play a critical role in this process ((50)). One other enabling is that fundamental genome instability and mutation occurs with disruption of DNA repair. In the same paper summarizing the next generation of

the hallmarks, Hanahan and Weinberg focused on the fact that cancer is not just cancer cells ((49)). Rather, it includes blood vessels, the lymphatics, the associated endothelial cells and pericytes, the immune inflammatory cells, and the associated fibroblasts. The notion that the primary tumor becomes invasive after it establishes its ability to metastasize, associated with the epithelial to mesenchymal transition (EMT), is something that's very sympathetic to the very recent data emerging ((51)). There are a series of complex interactions between these tumor-promoting inflammatory cells which both produce factors that act on cancer cells but then cancer cells similarly produce factors that act on the inflammatory cells. Recent findings (reviewed in (52)) suggest that tumor autophagy, induced by cytotoxic agents, releases HMGB1, IL1β, and the critical ATP to promote recruitment of dendritic cells and T-cells as part of an immunogenic response. The critical requirements for enabling successful immunotherapy of cancer have recently been summarized by experts in the field (53).

David Lyden performed interesting experiments defining the so-called pre-metastatic niche. The experiment was rather simple. What he did is he took a mouse, irradiated it and thentransplanted eGFP (green) bone marrow cells from another animal. These animals were then injected in the flank with eRFP (red) tumor cells and then observed the sequence of events leading to lung metastases. Approximately 9 days before frank tumors were found in the lung were foci of ‘green’ bone marrow derived inflammatory cells that he called the pre-metastastic niche. These cells were able to serve as a niche, the tumor essentially creating the soil distally that the seed could migrate into. This is a novel notion which has now been observed not just in the lung but also within the lymph node and in the liver and in the bone marrow where the pre-metastatic niche has been also been identified. On day 14 you have myeloid-derived or bone marrow-derived cells that arise, and then once they're established then you have migration into these sites of the tumor cells and their establishment. And he's gone on to demonstrate what some of the factors are, and although VEGF and expression of VEGF receptors were initially seen as being critically important in the early explanation of this strategy, subsequent investigation suggests that it's not all that simple. Indeed, Andrew Rhim’s paper suggests in a spontaneous epithelial tumor model that premalignant epithelial cells can delaminate and travel to lymph nodes where they presumably die a necrotic death and recruit inflammatory cells suitable for providing the niche ((51)). A nice review paper from Lyden's group ((54)) suggests that there are a number of factors, not just VEGF-A but TNF, SDF1, a chemokine, PIGF, and then a series of what are called DAMP’s (Damage Associated Molecular Pattern Molecules) including the granulocyte derived S100-A8 and A9 molecules. The prototypic DAMP, HMGB1, serves not only to promote quality control in mitochondria ((50)), but to also promote ‘programmed cell survival’ or autophagy in cells when stressed ((55)), limiting the ability of p53 to promote apoptosis in the cytosol ((56)).

Eric Lagasse a scientist at the University of Pittsburgh did an important experiment where he took animals who had failing livers and injected normal hepatocytes into the peritoneal cavity of these animals and the animals, not dying as they normally would, instead survived ((57)). And when he looked–because they were again fluorescently labeled – instead of going into the liver, they were found to migrate into the lymph nodes. He could establish, unlike in the gut, that in the mesenteric lymph nodes that normal hepatocytes could go into the lymph node and grow productively. And he reasoned that essentially the lymph node is just a factory for T cells and B cells but it also had an extracellular microenvironment suitable for hepatocytes to grow. He's gone on to show that he can do the same thing and get other normal cells, islets and thymic epithelial cells to grow in a lymph node.

And so we were challenged earlier to say what is cancer, but here is something that is normal cells able to grow in lymph node structures. And I was struck by some of the earlier findings that there's no direct relationship between finding these immunohistochemistry

positive cells in the lymph node and outcome for some of our patients. And even normal cells can find their way into lymph nodes and liver ((51)). Within the mesenteric lymph nodes, and so-called fatty or milky patches in the omentum, one observes hepatocytes growing productively inside the lymph nodes. It's really quite extraordinary.

Cancer in adults–not in children—appears to be the end stage of chronic inflammation. There are very few cancers that I'm aware of that in adults don't arise in the setting of chronic inflammation. But one of the ways we know that is that we find inflammatory cells–both dendritic cells and T cells and NK cells–in virtually all of these tumor types. And in virtually every instance more T cells, more dendritic cells is associated with a better prognosis. Fewer T cells and dendritic cells are associated with a worse prognosis ((58)). Pediatric cancers on average compared to the adult cancers, have very few T cells, almost no dendritic cells, but are full of macrophages. This suggests that pediatric cancers are an entirely different biology than adult cancers ((59)).

Charles Janeway, along with a talented Russian postdoc, Ruslan Medzhitov, was able to identify the so-called toll-like receptor 4 (TLR4) –that interacted with a Gram negative bacterial product LPS that we now know is prototypic of a whole series of TLRs that are important for Gram positive and Gram negative recognition as well as pathogenic viruses. And those are called pathogen associated molecular pattern molecules (PAMPs). But what is it that is associated with inflammation when it's sterile, when you have a heart attack, when you have a stroke, when you have a surgical procedure, when you have a cancer? And for want of a better term we have by homology called these molecules DAMPs, damage associated molecular pattern molecules, which are molecules expressed or released that are normally unavailable to the immune system but are released and recognized by immune cells following tissue injury. So what are these DAMPs? What are the damage associated molecular pattern molecules that provoke an inflammatory response?

This is actually a large number of intracellular constituents that are released when cells die the wrong way. And this includes the IL-1 family members, IL-1 alpha and beta; a new member which I think is going to be quite important, IL-33; HMGB1, high mobility group B1; cytochrome C; heat shock proteins; uric acid, the cause of gout; ATP; s100 proteins; LDH which you all know; and as well as very interestingly, mitochondrial DNA ((60)).

All cancer clinicians have been taught from the moment they initiated their careers that necrosis was a bad thing. Because the tumors that grew so quickly that they necrosed outgrew their blood supply but in actual fact we think it's because they released DAMPs that promote paradoxically reactive angiogenesis, stromagenesis and recruitment of all these calls that we've been hearing about today. And in virtually every tumor type that's been carefully examined with *P* values with a lot of zeros after them more necrosis is associated with a worse prognosis. There's one other factoid that I'd like to leave you with, which is the complexity of cancer biology. And that has to do with how cells die. It used to be thought that apoptosis was something that tumor cells just suppressed and that clever as they are to over-express Bcl-2, Bcl-XL, IAPs or survivin and mutate p53 to avoid apoptosis, there are now three articles–one from our group and one from two other groups appearing in *Nature* and *Science* respectively–showing that early in cancerogenesis if you block apoptosis either by getting rid of FAS or by getting rid of a downstream signaling molecule PUMA that is induced by p53, that you actually limit apoptosis and limit cancer development (reviewed in (61)). So at the beginning of cancer development you need heightened apoptosis and then replacement of the remaining epithelial cells. And late in tumor development you need to block apoptosis. And so basically our current feeling is that cancer treatment leads to dying tumor cells and unless you get the very last one, they release DAMPs such as HMGB1,

within the tumor microenvironment, interacting with cognate receptors driving proliferation, inflammation and a process known as autophagy, thereby promoting tumor growth.

As tumors progress they go from normal cells to preneoplastic cells to frankly invasive cancers with increase in both growth factor signaling and stress signaling. During this period your mode of cell death switches from balanced apoptosis and autophagy and essentially no necrosis to one associated with diminished apoptosis, enhanced autophagy, and when the cell must die, it necroses and releases DAMPs including HMGB1 promoting cycles of autophagy followed by necrosis and almost no apoptosis. So in conclusion, DAMPs and HMGB1 are links between inflammation and necrotic cell death.

Discussion

Donald L. Morton

The significance of micrometastasis in the sentinel node in different diseases has been investigated. First, the treatment approaches to breast cancer and melanoma are different. Every woman with breast cancer has a breast salvage procedure; she receives radiation to the breast and axilla, and she receives chemotherapy. Both of these modalities may sterilize any residual micrometastases in remaining axillary nodes. By contrast, in melanoma the only effective treatment we really have for nodal metastases is surgery.

Are micrometastases “real” metastases? In MSLT-I, patients stratified by Breslow thickness were randomized to wide excision plus sentinel node biopsy, with complete lymphadenectomy for sentinel node metastases, or to wide excision plus nodal observation. We found that with extended follow-up the observation group had a 20 percent incidence of nodal recurrence, the same as the rate of occult nodal metastasis identified by sentinel node biopsy. However, although the incidence of nodal metastasis was equal in the two treatment groups, disease-free survival was significantly longer in the sentinel node biopsy group. In addition, depending on the thickness of the primary, there was a treatment-related difference in distant disease-free metastasis. This difference disappeared for patients whose primary was ≥ 3.5 mm. This is not surprising because thicker primary melanomas would be more likely to spread quickly beyond the regional lymph nodes. In other words, even very small metastases in the sentinel node are functionally significant.

I also would like to comment on the immune status of the sentinel node. The sentinel node is highly immunosuppressed as compared to nonsentinel nodes in the same basin. Both tumor-positive and tumor-negative sentinel nodes have reduced numbers of dendritic cells. These dendritic cells are not mature (no dendrites), there are fewer T cells, and there are elevated levels of messenger RNA for IL-10. The profound immunosuppression in the sentinel node is related to tumor burden at the primary site, and it can be reversed by complete wide excision of the primary. The message is that lymph node metastases can and will metastasize if they are not removed. Early removal of nodal metastasis will result in cure of patients whose primary melanomas are thinner than 3.5 millimeters; patients with primary tumors thicker than 3.5 millimeters will have a difference in nodal recurrence but not in distant survival.

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