What Are the CD34+ Cells in Benign Peripheral Nerve Sheath Tumors?

Double Immunostaining Study of CD34 and S-100 Protein

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Abstract

To determine whether CD34 expression in nerve sheath lesions was found in a unique cell population or in a subset of nerve sheath cells, we performed double immunohistochemical staining using a standard avidin-biotin complex method with 2 separate color developing systems. We studied 40 neurofibromas and 16 neurilemomas. All lesions strongly expressed S-100 in nuclei and cytoplasm. CD34 was detected in cells having ameboid dendritic cytoplasm present in greatest numbers in Antoni B zones of neurilemomas, myxoid zones of neurofibromas, at the periphery of lobules in both tumor types, and condensed in apposition to perineurium. The CD34+ cells also were detected in normal nerves. They were infrequent in Antoni A zones of neurilemomas. No dual S-100 and CD34 expression was seen. This double immunostaining confirms the presence of a CD34-reactive non-Schwannian cell type in these neural neoplasms. The CD34+ cells also were detected in normal nerves. They were infrequent in Antoni A zones of neurilemomas. No dual S-100 and CD34 expression was seen. This double immunostaining confirms the presence of a CD34-reactive non-Schwannian cell type in these neural neoplasms. As the CD34+, S-100–negative cell population is present also in normal nerves and infrequently seen in the areas of cellular neoplastic Schwann cells, CD34+, S-100–negative cells in peripheral nerve sheath tumors most likely are nonneoplastic and may have a supportive function.

CD34 is a transmembrane glycoprotein expressed by human hematopoietic progenitor cells of lymphoid and myeloid lineage.1 When anti-CD34 antibodies are applied to paraffin-embedded tissue sections, however, they also react with normal vascular endothelial cells and perineural spindle cells and dermal dendritic cells.2 Certain leukemias, benign and malignant vascular tumors, and dermatofibrosarcoma protuberans are typically immunoreactive with antibodies against CD34. Other apparently unrelated neoplasms, such as lipomatous tumors3 and solitary fibrous tumors, are also CD34+.4 It is now believed that CD34 is encoded by a gene on chromosome 1q.5 The extracellular domain of its molecule is involved in the regulation of proadhesive and antiadhesive cellular behavior, while the intracellular domain probably functions in signal transduction.6

It has been suggested that CD34 also defines a normally occurring population of dendritic cells within the endoneurium of peripheral nerves that is distinct from fibroblasts and conventional Schwann cells.7 Similar cells were recognized in benign peripheral nerve sheath tumors with various proportions depending on the cellularity of the respective lesions. To confirm the presence of CD34+ cells and to exclude the possibility that these are of Schwannian origin, we applied a double immunohistochemical staining technique with 2 separate color developing systems to a series of benign peripheral nerve sheath tumors.

Material and Methods

Tissue and Patients

One hundred eight patients with benign peripheral nerve sheath tumors (74 neurofibromas and 34 neurilemomas) were
treated at the Health Sciences Center, Memorial University of Newfoundland, St John’s, between January 1988 and December 1997. Medical charts, surgical pathology reports, H&E-stained slides, and paraffin blocks were available for only 93 cases. All slides were reviewed by one of us (N.I.) to confirm the diagnosis and to assure the presence of ample embedded tissue remaining in the block. Cases with occasional bizarre cells, areas of necrosis, or increased mitotic activity were excluded. Adequate representative blocks of 56 peripheral nerve sheath tumors (40 neurofibromas and 16 neurilemmomas) were finally identified and included in the present study.

Immunohistochemical Methods

Double immunohistochemical staining was performed using a standard avidin-biotin complex method with 2 separate color developing systems. S-100 immunostaining first was performed using an autoimmunostainer (Tekmate 1000, Ventana, Tucson, AZ) using peroxidase as the enzyme and diaminobenzidine as the chromogen (brown staining). Then, CD34 immunostaining was performed by hand using an alkaline phosphatase development kit (Zymed, South San Francisco, CA) using alkaline phosphatase as the enzyme and fast red as the chromogen (red staining). Dilutions of CD34 antibody (HPCA-1, clone My10, Becton Dickinson, San Jose, CA) and of S-100 (polyclonal, code 7-311, lot 026, DAKO, Carpinteria, CA) were 1:25 and 1:2,500, respectively. Both antibodies were applied after heat-induced antigen retrieval.

Results

The demographic data and clinical features are summarized in Table 1. Immunoreactivity to both antibodies did not vary by patient age or sex or tumor topography. The morphologic features of the immunopositive cells, the pattern of staining, and the distribution of positive staining were different for each of the 2 markers Table 2, Image 1, and Image 2. No dual CD34 and S-100 expression was noted in any of the cases.

S-100 Immunoreactivity

Strong diffuse S-100 staining of elongated spindle cells was noted in every case. These cells were present in short bundles and interlacing fascicles. Their nuclei were slender, slightly twisted, and darkly stained. Cytomorphologically and immunophenotypically, these were neoplastic Schwannian cells.

CD34 Immunoreactivity

A distinct subpopulation of ameboid CD34+ cells was seen consistently in every tumor. However, there was great variability in the proportions of immunoreactive cells from one tumor to another. These cells featured relatively long dendritic cytoplasmic processes. Their nuclei were bland and round to oval and contained delicate chromatin. The distribution of these cells in both tumors is shown in Table 2.

Discussion

A subpopulation of CD34+ cells has been described in peripheral nerves, nerve sheath tumors, and related lesions. Since committed collagenous fibroblasts are CD34−, it was concluded that this subpopulation was unlikely fibroblastic. Topographically and cytomorphologically, Schwann cells and CD34+ cells were different. We tested the latter observation further by means of a double immunohistochemical staining technique. Since no dual CD34 and S-100 immunoreactivity was seen in any of the cells, we confirmed that Schwann cells and CD34+ cells are distinct cell types.

The fact that no CD34 ligand has been identified to date has excluded a hypothetical “receptor” function for this
transmembrane molecule. Molecular cloning sequencing and chromosomal localization studies of the CD34 gene also have failed to support a potential growth factor receptor role. On the other hand, the observation that CD34 is a highly negatively charged sialomucin-like glycoprotein has prompted the hypothesis that this molecule might be involved in the cell-cell or adhesive interactions. This hypothesis is further supported by the ultrastructural observation of its preferential localization on the luminal interdigitated microprocesses of adjacent endothelial cells in vivo. Our understanding of the functional role of the CD34 molecule has been enriched by in vitro studies focusing on endothelial cell response to various cytokines known to modulate cell cycling, adhesion, migration, and morphologic features. It was found that expression of CD34 at the antigen and messenger RNA levels was rapidly down-regulated by cytokines that also up-regulated adhesion molecules. This reciprocal pattern of regulation suggests that CD34 might enable expressive endothelial cells to proliferate and mobilize for wound healing and inflammatory repair.

CD34+ cells have been identified in many nonvascular mesenchymal lesions, including adipose tissue tumors, dermatofibrosarcoma protuberans, giant cell angiofibroma, stromal tumors of the gastrointestinal tract, atypical decubital fibroplasia (ischemic fasciitis), and solitary fibrous tumors. However, in gastrointestinal stromal tumors, an emerging literature demonstrates that a significant number of tumors also express an antigen associated with interstitial cells of Cajal using CD117 (c-kit protein). In the case of CD34 in neural lesions, Weiss and Nickoloff postulated that these cells might have a supportive role for Schwann cells. The authors noted that an analogous situation occurs in the bulge portion of the hair shaft, which is the site of proliferative activity. In this location, CD34+ cells have a supportive role conducive to further proliferation of adjacent stem cells. In peripheral nerve sheath tumors and in other neoplasms, it would be important for a proposed supportive subpopulation of cells to be able to mobilize to better serve the proliferating neoplastic cells. The recently identified biochemical and molecular evidence pointing to the role of CD34 molecule as one of the regulators of cell adhesion thus may be complementary to the proposed supportive function of CD34+ cells. Certainly one might expect that some neoplasms are composed de novo of such cells and, in fact, Suster and Fisher suggested that spindle cell lipoma may represent a dendritic cell neoplasm occurring in fat rather than an adipocyte tumor.

Another interesting aspect is that CD34 expression by endothelial cells is not constitutive but regulated, possibly by cell contact, proliferation, or changes of the extracellular environment. In vitro studies showed that CD34 expression was lost progressively with cell cycling and could not be rescued after 9 population doublings. This down-regulation thus may be associated with cell senescence in which “younger” lesions exhibit more reactivity than “older” lesions. This may explain why CD34 staining in our cases...
varied from one case to another. The variability in the prevalence of CD34+ subpopulations in peripheral nerve sheath tumors has been observed by others.7

The present study confirms the presence of a CD34+, S-100-negative dendritic, non-Schwannian cell subpopulation in normal nerves and in benign peripheral nerve sheath tumors. These cells are uniformly present in tumors from various body sites, and their distribution is not related to that of Schwann cells. They are most likely nonneoplastic and may serve supporting functions.

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