**Identification Of Novel Pigmented Villonodular Synovitis Surface Markers Absent On Surrounding Intra-articular Tissues.**

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Introduction: Pigmented villonodular synovitis (PVNS) is a benign but locally aggressive proliferative disease, thought to arise from synovial fibroblasts. This tumor affects large joints, where 75% of cases occur in the knee, though may also occur in the hip, ankle, shoulder and elbow. The disease presents on average between 30-40 years, ranges from 18-77 years, and both genders are equally affected. Despite the low incidence of this tumor in the US (1/500,000) this disease can be particularly devastating for young patients, who may develop disabling arthritis requiring a joint replacement or in some cases even amputation. PVNS rarely metastasizes, and due to the proximity of these tumors close to or within joints, marginal resection is the preferred treatment. However, PVNS has a high frequency of local recurrence, and often patients require multiple surgical procedures over their lifetime. Due to the significant morbidity and reduced quality of life for PVNS patients, there is a significant need to characterize the etiology of this disease and develop non-surgical targeted therapies. The aim of this study was to identify novel and specific surface markers for PVNS, and gain insight into PVNS pathogenesis.

Methods: The samples used to identify PVNS surface markers include PVNS tumors (n=3), intra-articular joint tissues including anterior cruciate ligament (ACL; n=2), bone (n=4), cartilage (n=4), synovium (n=4), and also fibroblasts (n=4) as a non-articular tissue control. RNA was extracted from these tissues and reverse transcribed into complementary DNA (cDNA). Gene expression profiling for 69 surface markers was performed using relative quantitative real-time PCR technique. Hierarchical clustering and marker selection was performed using the publically available GENE-E software (www.broadinstitute.org).

Results: Overall, PVNS has a similar surface profile to that of ACL, bone and synovium (Figure 1a). Despite these similarities, hierarchical clustering analysis revealed that PVNS tumors distinctly cluster independently of bone and synovium, indicating substantial gene expression differences (Figure 1a). Candidate marker selection analysis for PVNS compared to all other tissues revealed two candidates, CD68 and the novel marker CD226. As shown in Figure 1b, CD68 and CD226 are expressed the greatest in PVNS compared to other tissue. Further validation of these markers is required to establish the role of CD68 and CD226 in PVNS, and whether these markers are suitable therapeutic targets.

In addition to the identification of novel markers to be used for therapeutic targeting of PVNS, the cell surface markers investigated here maybe used distinguish PVNS from synovium and other tissues. As shown in Figure 1c, as few as 10 markers can be used to cluster PVNS tumors into a separate group from synovium, and these markers from fibroblasts, chondrocytes, ACL, and bone.

Discussion: This is the first study characterizing the gene expression of 69 surface markers in PVNS tumors. The results of this study revealed that PVNS tumors have a distinct surface profile compared to other healthy intra-articular tissues. This study identified two candidate markers for PVNS, CD68 and CD226. Identification of CD68 in PVNS supports previous studies that suggest PVNS may originate from synovium (Ravi et al. 2011), as CD68 is a marker for synoviocytes. In addition, CD68 is a marker for giant cells, which are also present in PVNS tissues. Furthermore, Berger and colleagues (2004) identified that both diffuse and localized PVNS proliferating synoviocytes were CD68+, where as non-proliferating synovial tissue were low/negative for CD68. Local targeting of CD68+ PVNS cells may provide a novel treatment for patients, as CD68 is not expressed on bone and variably expressed in cartilage and synovium, which would indicate a reduced risk for off-target effects.

CD226 is expressed on T cells NK cells, platelets, and monocytes, and mediates cellular adhesion and also differentially regulates the proinflammatory (Th1/Th17)/anti-inflammatory balance (Th2) (Lozano et al 2013). There have been no studies of CD226 in PVNS to date. In systemic sclerosis, this surface marker has been found to be critical for T cell mediated inflammation-driven dermal fibrosis in a systemic sclerosis mouse model (Avouac et al. 2013). PVNS tumors contain a chronic inflammatory cell infiltrate (Oehler et al. 2000), therefore there may be possible that a CD226 mediated inflammatory mechanism may be involved in PVNS etiology. The results of this study cannot discriminate whether CD226 was expressed on synoviocytes or immune cells, however CD226 is overexpressed in PVNS compared to other tissue times, and is a good candidate for further investigations. It is also interesting to note that anti-CD226 neutralizing antibody was able to protect bleomycin-induced fibrosis in the systemic sclerosis mouse model, and no serious adverse events were observed (Avouac et al. 2013).

Currently there are no clinical tools to distinguish PVNS from surrounding tissues. The high recurrence rate of PVNS following marginal resection indicates that the tumor is not completely removed on the initial surgery. To reduce the recurrence date of...
PVNS, expression of cell surface markers may be used to develop antibody panels that would allow pathologist to determine whether the resection is successful. From the gene expression profiling performed in this study, 10 markers were found to discriminate PVNS from surrounding tissues. Clinical development of this tool may be highly relevant for future treatment of PVNS.

In summary, our study aimed to identify novel and specific surface markers for PVNS, and gain insight into the pathogenesis of PVNS. This studied identified two candidate markers CD68 and CD226, which are overexpressed in PVNS tissue compared to healthy surrounding intra-articular tissues. Further characterization of these markers and their role in PVNS pathogenesis, could lead to the development of non-surgical interventions for this debilitating disease.

**Significance:** PVNS is a proliferative tumor that arises in joints including the knee, hip and ankle. The uncontrolled growth of this tumor causes pain and swelling of the joint, and eventually results in damage to the bone and cartilage of the joint. The morbidity of this disease is significant, particularly as this disease can occur in individuals as young as 18 years old. Since this tumor does not metastasize, the best treatment currently available for PVNS is surgery. However, the recurrence rate of this tumor after surgery is 50%, and thus PVNS patients suffer a lifelong battle with this tumor. Therefore, there is a significant need to develop non-surgical treatments that could manage the disease and prevent or delay the need for surgical resection.

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**References:**

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Figure 1: Surface marker expression profiling of PVNS.
(A) Gene expression profiling for surface markers. (B) CD28 and CD26 are marker markers for PVNS. (C) 10 surface markers can be used to distinguish PVNS from synovial and other tissues.