


Canonical Wnt/ β -catenin signaling activation in soft-tissue sarcomas: A comparative study of synovial sarcoma and leiomyosarcoma

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Abstract

Background: Previous studies have shown that aberrant activation of the Wnt/ β -catenin pathway is associated with many malignant neoplasms. This includes some soft-tissue sarcoma phenotypes, most notably synovial sarcoma, implicating potential targets for novel molecular therapies.

Objective: We investigate the level of Wnt/ β -catenin pathway activation present in leiomyosarcomas relative to synovial sarcomas, using expression of LEF1 and β -catenin as surrogates.

Methods: Cancer outlier profile analysis was performed on messenger RNA expression datasets in OncoPrint (70 synovial sarcomas, 178 leiomyosarcomas). Results for LEF1 and β -catenin messenger RNA expression were reported in terms of median-centered intensity. Separate immunohistochemical studies were performed on tissue microarrays created from 77 synovial sarcomas and 89 leiomyosarcomas using antibodies to LEF1 and β -catenin. Tumors with unequivocal strong nuclear staining involving $\geq 5\%$ of cells were interpreted as positive.

Results: Cancer outlier profile analysis demonstrated a higher level of LEF1 messenger RNA expression in synovial sarcomas than in leiomyosarcomas ($p < 0.0001$), but showed no significant difference in β -catenin messenger RNA expression ($p = 0.868$). Immunohistochemistry showed most synovial sarcomas had strong nuclear expression of LEF1 (79%) and β -catenin (84%), while a small minority of leiomyosarcomas had strong nuclear expression of LEF1 (5%) and β -catenin (6%).

Conclusion: These results provide further evidence that aberrant activation of the Wnt/ β -catenin pathway is present in most synovial sarcomas, but not in most leiomyosarcomas. While targeting the constituents of this pathway might be effective in the treatment of synovial sarcomas, it is not likely to be an effective strategy in the treatment of leiomyosarcomas.

Keywords

Wnt pathway, LEF1, synovial sarcoma, leiomyosarcoma, soft tissue neoplasm, pathology, beta-catenin

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Introduction

Wnt signaling plays a critical role in many biologic processes, including (among others) cell fate determination, neural patterning, organogenesis, and homeostasis. As a consequence of its importance in cellular and organismal development, Wnt signaling consists of highly regulated and evolutionarily conserved networks of signal transduction cascades. One pathway of particular interest is mediated by β -catenin and is referred to as the canonical Wnt pathway. Under normal circumstances, β -catenin is targeted and degraded by a complex of cytoplasmic proteins which include adenomatous polyposis coli (APC), axis inhibition protein (AXIN), glycogen synthase kinase 3 (GSK3 β), and casein kinase 1 (CK1). However, the binding of specific secreted glycoproteins (collectively referred to as Wnt ligands) to the extracellular domains of Frizzled and LRP5/6 receptors initiates a cascade of events which disrupts the aforementioned protein complex and ultimately prevents the degradation of β -catenin. As a consequence, β -catenin accumulates in the cytoplasm and is subsequently translocated into the cell nucleus, where it interacts with members of the T cell factor (TCF) and lymphoid enhancer-binding factor (LEF) families to alter gene expression.^{1,2}

The dawn of the molecular era of medicine in the 1990s and early 2000s led to the realization that various aberrancies in the canonical Wnt pathway are associated with certain types of malignancies.² One of the first genetic alterations to be associated with cancer was mutation of the APC gene in patients with the hereditary colorectal cancer syndrome familial adenomatous polyposis (FAP).²⁻⁴ Loss of APC function (as one of the major proteins of the β -catenin degradation complex) leads to the inappropriate accumulation of β -catenin, which subsequently drives aberrant gene expression and cellular proliferation. Since then, many different types of malignancies have been associated with genetic alterations leading to aberrancies in the canonical Wnt/ β -catenin pathway: some hepatocellular carcinomas, pancreatic carcinomas, adrenocortical carcinomas, melanomas, and several others.²

The majority of previously described malignancies associated with aberrancies in the canonical Wnt pathway are carcinomas (i.e. malignancies of epithelial cell origin). However, several expression-profiling studies have demonstrated a link between upregulation of the Wnt/ β -catenin pathway and the tumorigenesis of synovial sarcoma, a malignant mesenchymal spindle cell neoplasm characterized by the reciprocal translocation t(X;18) (p11;q11) with fusion of SS18 (SYT) to SXX1, SXX2, or (rarely) SXX4.^{1,5-9} These studies have guided ongoing clinical trials by targeting various constituents in the canonical Wnt pathway in the quest to develop effective molecular therapies in patients with synovial sarcoma.^{1,10-12} Interestingly, relatively recent studies published by Vijayakumar et al.¹³ demonstrated that approximately 50% of tumors analyzed from 12 distinct

human sarcoma subtypes exhibited upregulated autocrine canonical Wnt signaling. In three of four leiomyosarcomas that were studied, Vijayakumar et al. reported demonstrating the presence of upregulated Wnt signaling activity using β -catenin and TCF as surrogates.¹³ Like synovial sarcoma, leiomyosarcoma is an aggressive malignant mesenchymal spindle cell neoplasm of soft tissues. However, unlike synovial sarcoma, leiomyosarcoma is characterized by smooth muscle differentiation and a complex karyotype without any known disease-defining genetic alterations.^{14,15} To date, little is known about the tumorigenic mechanisms of leiomyosarcoma.

In this study, we investigate whether the Wnt/ β -catenin pathway is upregulated (as determined through LEF1 and β -catenin messenger RNA (mRNA) expression and nuclear protein expression) in leiomyosarcoma relative to synovial sarcoma in hopes of predicting whether the types of novel molecular therapies (targeting constituents of the canonical Wnt pathway) currently under investigation for synovial sarcoma may have a potential role in the future treatment of leiomyosarcoma.

Materials and methods

Cancer outlier profile analysis

The online Oncomine database (www.oncomine.org) and described by Rhodes et al.¹⁶ was searched for Affymetrix mRNA expression datasets pertaining to synovial sarcoma and leiomyosarcoma. Cancer outlier profile analysis (COPA) described by Tomlins et al.¹⁷ was performed using either LEF1 or β -catenin as filters and the results were expressed in terms of median-centered intensity. The synovial sarcoma and leiomyosarcoma cases were compared for overall level of LEF1 mRNA expression and subsequently β -catenin mRNA expression. A *p* value ≤ 0.05 was considered significant.

Tissue microarrays and immunohistochemistry

Tissue microarray (TMA) slides were obtained from the University of Michigan pathology archive. The TMAs were previously constructed from 77 synovial sarcomas and 89 leiomyosarcomas (29 uterine and 60 non-uterine) between January 1990 and April 2007 with approval from the University of Michigan Institutional Review Board. Each TMA consisted of 1.0 mm formalin-fixed, paraffin-embedded neoplastic tissue cores in triplicate with a variety of normal tissues used as controls.

Immunohistochemistry (IHC) was performed on the TMA sections using a Ventana Autostainer Link (Dako, North America, Carpinteria, CA). For the LEF1 immunostaining, rehydrated sections were pretreated with heat-induced epitope retrieval performed with FLEX TRS Low pH Retrieval buffer (6.1) for 20 min. After peroxidase blocking, LEF1 rabbit monoclonal antibody (EPR2029Y,

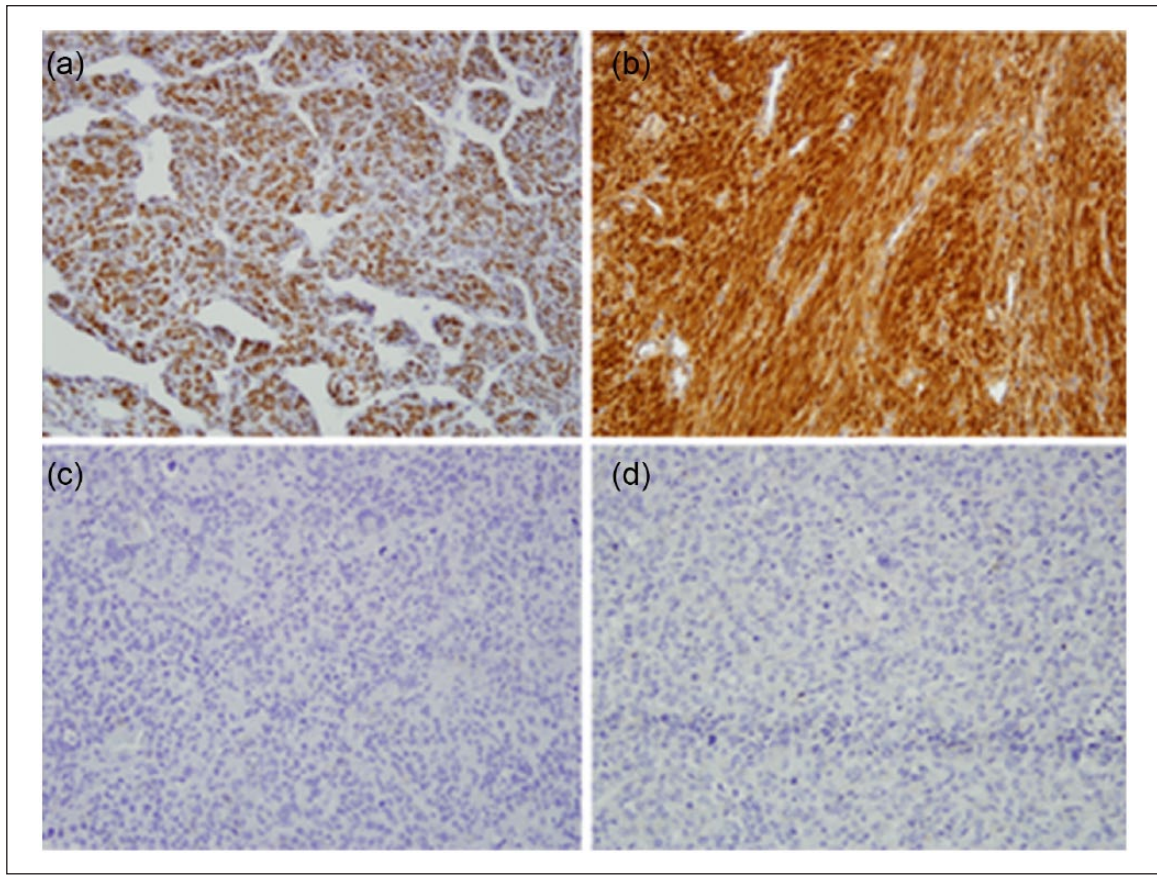


Figure 1. Examples of LEF1 and β -catenin immunohistochemical expression in synovial sarcoma and leiomyosarcoma. Strong unequivocal (score = 2+) nuclear staining for (a) LEF1 and (b) β -catenin in synovial sarcoma. Negative (score = 0) nuclear staining for (c) LEF1 and (d) β -catenin in leiomyosarcoma. Cytoplasmic staining with lack of 2+ nuclear staining was interpreted as negative.

Abcam, Cambridge, MA) was applied at a dilution of 1:250 at room temperature for 60 min. For the β -catenin immunostaining, rehydrated sections were pretreated with heat-induced epitope retrieval performed with FLEX TRS High pH Retrieval buffer (9.01) for 20 min. After peroxidase blocking, the β -catenin mouse monoclonal antibody (14/ β -Catenin, BD Transduction Laboratories, San Jose, CA) was applied at a dilution of 1:500 at room temperature for 30 min. The FLEX HRP EnVision System was used for detection with both antibodies. DAB chromagen was then applied for 10 min. Slides were counterstained with Harris Hematoxylin for 5 s and then dehydrated and coverslipped.

Two authors (L.M.B. and D.R.L.) independently reviewed each tissue core to assess the presence of adequate neoplastic cells and to interpret the level of nuclear expression of LEF1 and β -catenin. IHC staining intensity was scored as 0, 1+, or 2+. Tumors with unequivocal 2+ nuclear staining involving $\geq 5\%$ of cells were interpreted as positive. Tumors with $< 5\%$ of cells exhibiting 2+ nuclear staining (regardless of cytoplasmic staining) were interpreted as negative (Figure 1). Statistical analysis included calculation of mean, median, and standard deviation to illustrate percentages of positive (2+) nuclear staining for each antibody among tumor types.

Results

COPA

Our search of the online Oncomine database yielded a total of 11 expression datasets, representing 70 cases of synovial sarcoma (4 datasets) and 178 cases of leiomyosarcoma (7 datasets), which were included in our COPA. The results of this analysis were expressed in terms of median-centered intensity and represented in graphical format as depicted in Figure 2. Overall, there was a significantly higher level of LEF1 mRNA expression in the synovial sarcoma cases when compared to the leiomyosarcoma cases ($p < 0.0001$). However, there was no significant difference between synovial sarcoma and leiomyosarcoma with respect to the level of β -catenin mRNA expression ($p = 0.868$).

IHC

A total of 77 cases of synovial sarcoma and 89 cases of leiomyosarcoma were stained with antibodies for LEF1 and subsequently for β -catenin (Table 1). However, 20 of the synovial sarcomas stained for LEF1, 21 of the synovial sarcomas stained for β -catenin, 11 of the leiomyosarcomas stained for LEF1, and 12 of the leiomyosarcomas stained for β -catenin

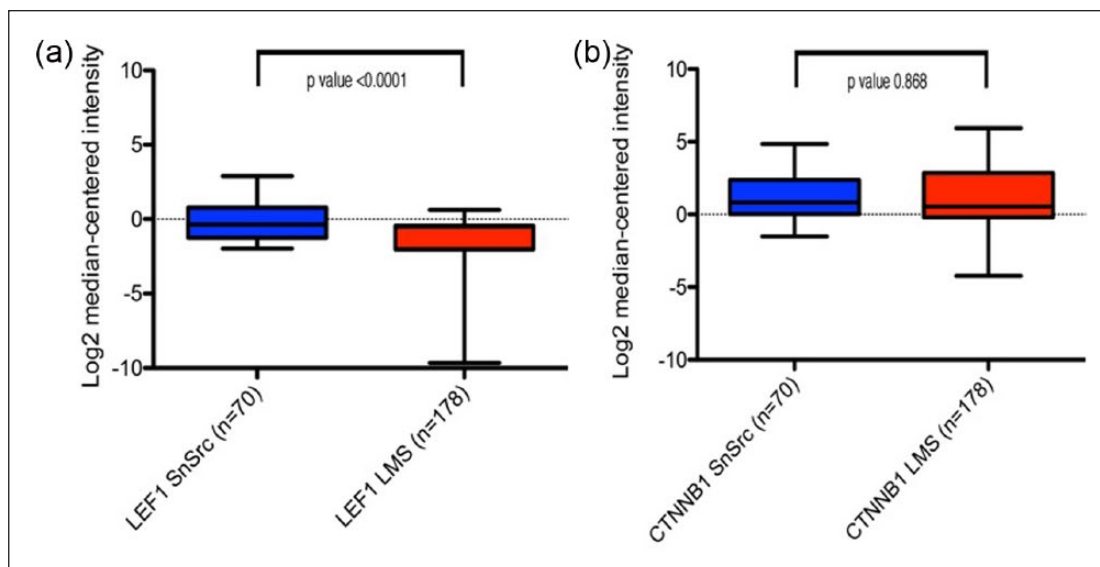


Figure 2. Cancer outlier profile analysis for (a) LEF1 and (b) β -catenin mRNA expression in synovial sarcoma and leiomyosarcoma. SnSrc: synovial sarcoma; LMS: leiomyosarcoma; CTNNB1: β -catenin.

Table 1. Immunohistochemical nuclear expression of LEF1 and β -catenin in synovial sarcoma and leiomyosarcoma cases reviewed at the University of Michigan.

Sarcoma phenotype	LEF1	β -Catenin
	Percent positive	Percent positive
Synovial sarcoma ^a	79% (<i>n</i> = 45 of 57)	84% (<i>n</i> = 47 of 56)
Leiomyosarcoma ^b	5% (<i>n</i> = 4 of 78)	6% (<i>n</i> = 5 of 77)

LEF1: lymphoid enhancer-binding factor 1.

^aA total of 77 synovial sarcomas were stained with LEF1 and β -catenin antibodies. However, 20 cases stained with LEF1 and 21 cases stained with β -catenin were excluded due to lack of adequate viable tumor cells.

^bA total of 89 leiomyosarcomas were stained with LEF1 and β -catenin antibodies. However, 11 cases stained with LEF1 and 12 cases stained with β -catenin were excluded due to lack of adequate viable tumor cells.

lacked adequate viable tumor cells for proper evaluation. These cases were therefore excluded from our study.

Immunostaining with the LEF1 antibody demonstrated strong unequivocal (2+) nuclear positivity in 45 of 57 synovial sarcomas (79% of tumors; mean 49% of nuclei per tumor, mode 95%, standard deviation 34%) and strong unequivocal (2+) nuclear positivity in only 4 of 78 leiomyosarcomas (5%). Immunostaining with the β -catenin antibody demonstrated strong unequivocal (2+) nuclear positivity in 47 of 56 synovial sarcomas (84% of tumors; mean 80% of nuclei per tumor, mode 90%, standard deviation 22%) and strong unequivocal (2+) nuclear positivity in only 5 of 77 leiomyosarcomas (6%).¹⁸

Discussion

The results of both COPA and immunohistochemical interrogation of our TMAs demonstrated a higher level of LEF1 expression in synovial sarcomas compared to

leiomyosarcomas. However, the results of COPA and IHC with respect to β -catenin initially appear to be discrepant: COPA showed no significant difference in β -catenin mRNA expression between the two sarcoma phenotypes, while IHC clearly demonstrated a higher level of nuclear β -catenin expression in the synovial sarcomas compared to the leiomyosarcomas. An explanation for this discrepancy is that the data we obtained from the Oncomine database and used in our COPA reflected overall mRNA expression (including both nuclear and cytoplasmic expression) of LEF1 and β -catenin, whereas for our TMAs we only considered strong nuclear immunohistochemical staining with LEF1 and β -catenin protein to be positive. Cytoplasmic staining without concomitant nuclear staining was interpreted as negative. In the canonical Wnt/ β -catenin pathway, only nuclear LEF1 and β -catenin proteins can drive gene expression and subsequently cellular proliferation. This requires translocation of these proteins into the cell nucleus. CTNNB1 expression at the mRNA level is not indicative of Wnt or β -catenin signaling, since β -catenin is regulated at the protein level, and the amount of global cellular β -catenin mRNA does not reflect the amount of β -catenin protein that is translocated into the nucleus. Therefore, while COPA showed that there was no significant difference between the two sarcoma phenotypes in terms of overall β -catenin mRNA expression, our immunohistochemical results indicate that there is a higher abundance of nuclear β -catenin protein capable of driving altered gene expression and aberrant cellular proliferation in synovial sarcoma compared to leiomyosarcoma.

With respect to synovial sarcoma, our results corroborate the findings of previous profile-expression studies showing that the Wnt/ β -catenin pathway is aberrantly activated in the majority of cases.⁵⁻⁹ This lends further

credence to the notion that the Wnt/ β -catenin pathway plays an important role in the tumorigenesis of synovial sarcoma. LEF1 is a robust biomarker of canonical Wnt activation regardless of precise cause of pathway.¹⁸ However, as others have previously suggested, targeting individual constituents of this pathway may prove fruitful in designing and discovering novel molecular therapies for the treatment of patients with synovial sarcoma. However, with respect to leiomyosarcoma, our results show that the β -catenin is not overexpressed in the cell nucleus. This is potentially at odds with a previous study published by Vijayakumar et al.,¹³ in which they demonstrated upregulation of the Wnt/ β -catenin pathway via detection of uncomplexed β -catenin in three of four leiomyosarcomas that they included in their study. The only possible explanation that we have for the discrepancy between our results and the results of Vijayakumar et al. is that they may not have distinguished between nuclear and cytoplasmic β -catenin protein expression. Nevertheless, our results are strengthened in that we analyzed a much larger cohort of cases ($n=89$). Our findings suggest that mechanisms other than aberrant Wnt/ β -catenin signaling more likely contribute to the tumorigenesis of leiomyosarcomas. Thus, the molecular therapies under investigation for the treatment of patients with synovial sarcoma are much less likely to yield effective results in the treatment of patients with leiomyosarcoma.

Conclusion

Soft-tissue sarcomas encompass a wide spectrum of different phenotypes. In contrast to this diversity, relatively little remains known about their pathogenesis. In part due to our very limited understanding of their biology, relatively few therapeutic options exist beyond surgery for most soft-tissue sarcomas. The few adjuvant and neoadjuvant therapies which do exist are the same for virtually all sarcoma phenotypes, despite their incredible diversity. The advent of molecular diagnostics has begun to reveal that despite their mesenchymal origins, not all sarcomas arise via aberrations of the same developmental pathways. The identification of genetic alterations, in general, has many potentially beneficial implications for patient care. The detection of such alterations can supplement histomorphologic impressions in making a diagnosis, potentially provide critical information regarding prognosis and anticipated clinical behavior of certain malignancies, identify tumorigenic mechanisms that can be targeted by molecular therapies for use in clinical trials, and/or play a crucial role in monitoring minimal residual disease by molecular methods in patients who are undergoing therapy. Given the limited extent of our collective understanding of the pathobiology of sarcomas (particularly in comparison to carcinomas and hematologic malignancies), there is much potential benefit to be gained from rigorous study in this area. One such example is the association of aberrant Wnt/ β -catenin signaling in the pathogenesis of synovial sarcoma, a piece of

information which may hopefully lead to the discovery of effective molecular therapies for patients with synovial sarcoma in the not too distant future. It is with this same rationale that the authors of this study believe it is important to pursue future large-scale profile-expression studies to elucidate the tumorigenic mechanisms of other soft-tissue sarcoma phenotypes through investigation not only of the Wnt/ β -catenin pathway but also other important developmental pathways implicated in cancer biology.

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Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from Evaluation of Biomarkers and Clinical Tumor Behavior in Soft Tissue and Osseous Sarcoma (Michigan Medicine IRB HUM00068553).

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Informed consent

Informed consent was not sought for this study because this was not a clinical trial.

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