Increased voltage-dependent K^+ channel Kv1.3 and Kv1.5 expression correlates with leiomyosarcoma aggressiveness

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Abstract. Voltage-dependent K^+ channels (Kv) are involved in the proliferation and differentiation of mammalian cells, since Kv antagonists impair cell cycle progression. Although myofibers are terminally differentiated, some myoblasts may re-enter the cell cycle and proliferate. Since Kv1.3 and Kv1.5 expression is remodeled during tumorigenesis and is involved in smooth muscle proliferation, the purpose of this study was to analyze the expression of Kv1.3 and Kv1.5 in smooth muscle neoplasms. In the present study, we examined human samples of smooth muscle tumors together with healthy specimens. Thus, leiomyoma (LM) and leiomyosarcoma (LMS) tumors were analyzed. Results showed that Kv1.3 was poorly expressed in the healthy muscle and indolent LM specimens, whereas aggressive LMS showed high levels of Kv1.3 expression. Kv1.5 staining was correlated with malignancy. The findings show a remodeling of Kv1.3 and Kv1.5 in human smooth muscle sarcoma. A correlation of Kv1.3 and Kv1.5 expression with tumor aggressiveness was observed. Thus, our results indicate Kv1.5 and Kv1.3 as potential tumorigenic targets for aggressive human LMS.

Introduction

Voltage-dependent potassium channels (Kv) contribute to the myogenic regulation of vascular tone (1). In addition, Kv channels act in the cell cycle progression and differentiation of smooth muscle cells (2,3). In this context, Kv channels govern cell proliferation by controlling specific checkpoints during the cell cycle that are crucial for further cycle progression (4-7). During the G1/S phase of the cell cycle a temporary hyperpolarization occurs, in which Kv channels are involved (8). The voltage-dependent Kv1.3 and Kv1.5 channels are involved in cell proliferation, and expression remodeling has been described during neoplastic growth (5,9). A number of tissues, such as brain, muscle and the immune system, co-express the two channels (10-12). Kv1.3 abundance is generally down-regulated, whereas Kv1.5 expression is enhanced in several types of human cancer (5,9,13).

Kv1.3 and Kv1.5 increase during myoblast proliferation (11). Kv1.5 is the main subunit in skeletal myoblasts, whereas Kv1.3 is critical in smooth muscle (2,3). Thus, Kv1.5 exhibits a cycle-dependent regulation in skeletal muscle myoblasts (11) and Kv1.3, which controls proliferation in leukocytes (14,15), is also involved in vascular cell proliferation and migration (2,3). In a preliminary report, it was noted that Kv1.3 and Kv1.5 undergo remodeling in human skeletal muscle sarcomas (13), which was recently demonstrated in depth (16), whereas there are no studies addressing Kv1.3 and Kv1.5 during neoplastic smooth muscle proliferation. The aim of the present study was to investigate, for the first time, the expression of Kv1.3 and Kv1.5 in human smooth muscle neoplasms. Leiomyoma (LM) and leiomyosarcoma (LMS) are benign and malignant soft tissue neoplasms, respectively, that arise from smooth muscle cells. LM is the most common type of uterine neoplasm occurring in women older than 35 years. Retroperitoneal LMS, a common primary retroperitoneal neoplasm, arises from smooth muscle within arteries, veins or the bowel.

In the present study, we observed a positive correlation between the malignancy of the smooth muscle neoplasm and the expression of Kv1.3 and Kv1.5. The results were notable for Kv1.3, which seems to be crucial in smooth myoblasts (2,3). The results indicate that Kv1.5 and Kv1.3 are potential tumorigenic targets for aggressive LMS.

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Table I. Sample characteristics and diagnostic markers.

Diagnosis	n	Mean age	Gender	Diagnostic method/markers
Retroperitoneal leiomyosarcoma (LMS)	3	67	M	Vimentin, desmin, smooth muscle actin
Uterine leiomyoma (LM)	3	43	F	Blinded morphometry

M, male; F, female.

Materials and methods

Patients, tissue characteristics and sample processing. A total of 6 smooth muscle tumor samples, 3 LM samples and 3 LMS samples were obtained from the Department of Pathology of Vall d'Hebron University Hospital and the Department of Pathology of Bellvitge Hospital (Barcelona, Spain) between 2008 and 2009. A summary of patient and sample information is shown in Table I. Four non-tumoral uterine smooth muscle samples were added to the series as controls. Patients were informed and gave their consent for sample collection. The research investigations were approved by the University and the hospital's ethics committee.

Control and tumor samples were fixed in neutral formalin and embedded in paraffin for immunohistochemistry. Blinded diagnosis was performed independently by two pathologists using light microscopy and conventional hematoxylin and eosin staining. Tissue microarrays were constructed, including 3 cores of 2-mm diameter from each case; two cores were from the central area of the tumor and the third was obtained from the invasive front. The results from representative cores were recorded.

For LMS, markers such as smooth-muscle actin, vimentin and desmin were used. LM was diagnosed with simple hematoxylin and eosin staining and light microscopy evaluation.

Antibodies and immunohistochemistry. Immunohistochemical staining using the avidin-biotin-peroxidase technique was performed for each antibody. Tissue microarrays were generated on poly-L-lysine-coated glass slides. Sections were deparaffinized in xylene and rehydrated in graded alcohol. For antigen retrieval, the microarrays were heated either in a pressure cooker in 10 mM citric acid monohydrate, pH 6.0, for 5 min (Kv1.5), or in 10 mM citric acid monohydrate, pH 9.0 for 40 min in a 98˚C water bath (Kv1.3). Endogenous peroxidase was blocked by incubating the sections in 3% hydrogen peroxidase blocking solution for 10 min. Arrays were immunoblotted with anti-Kv1.3 (1:70) and anti-Kv1.5 (1:100) polyclonal antibodies (Alomone) for 1 h, followed by an HRP-labeled polymer anti-rabbit antibody (DakoCytomation, Glostrup, Denmark) for 30 min. Immunohistochemical detection was performed with the EnVision system (DakoCytomation). The samples were counterstained with hematoxylin, dehydrated and mounted. Negative controls were prepared in all cases by omitting the primary antibody as described in previous studies (16,17). Antibody staining has been extensively validated previously (13,16,17). Additionally, as previously reported, but not yet understood, some Kv1.5 nuclear staining was observed (13,16-18); therefore, only cytoplasm staining was evaluated.

Immunohistochemistry evaluation and statistical analysis. Cases were evaluated by pathologists who assessed the percentage of positively stained cells and semi-quantitatively described the staining intensity. Kv1.3 and Kv1.5 staining was evaluated by calculating a histoscore (Hscore). The Hscore = (1 x % weakly stained cells) + (2 x % moderately stained cells) + (3 x % strongly stained cells), with results ranging from 0 to 300, as previously described (16,19). Weakly stained cells were marked (+), moderately stained cells (++) and strongly stained cells (+++). In randomly chosen cases, the immunostaining was performed in complete tissue sections to evaluate the consistency of the results.

Values were expressed as the means ± SEM and analyzed by Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Immunohistochemistry of Kv1.3 and Kv1.5 in smooth muscle tumors. In vascular smooth muscle (VSMC), Kv1.3 and Kv1.5 expression remodels during VSMC proliferation (2,3). Kv1.5 downregulates, whereas Kv1.3 increases (2). However, the expression of Kv1.3 and Kv1.5 in other smooth muscle tissues, such as the bladder or uterus, is under debate (3). Since the two channels are involved in cell proliferation, we investigated the expression of Kv1.3 and Kv1.5 in human smooth muscle neoplasms. Two types of human smooth muscle tumors were studied: an indolent uterus smooth muscle LM and an aggressive retroperitoneal LMS. Healthy specimen counterparts were also included.

Fig. 1 shows representative images of Kv1.3 and Kv1.5 staining in LM and LMS compared with healthy myometrial samples. Remodeling of Kv1.3 was detected in uterine smooth muscle biopsies. Kv1.3 staining in the control sample (Fig. 1A) was faint (+, 90%) and extremely low (-/+, 50%) in the less aggressive LM (Fig. 3C). However, the expression of Kv1.3 was clearly notable (++, 100%) in LMS (Fig. 3E). By contrast, Kv1.5 staining was almost absent (-, 98%) in the healthy smooth muscle (Fig. 1B). A heterogeneous faint Kv1.5 expression was detected (+, 50%) in LM samples (Fig. 1D), whereas LMS showed a homogenous but poor expression (-/+, 100%) of Kv1.5 (Fig. 1F).

Histoscore evaluation for smooth muscle tumors. Hscore has been validated as a semi-quantitative analysis for evaluation of the protein expression with immunohistochemistry (16,19). Therefore, to analyze Kv1.3 and Kv1.5 staining, an Hscore was calculated as described in Materials and methods (Fig. 2). Fig. 2A shows a graphical representation of the Hscore for

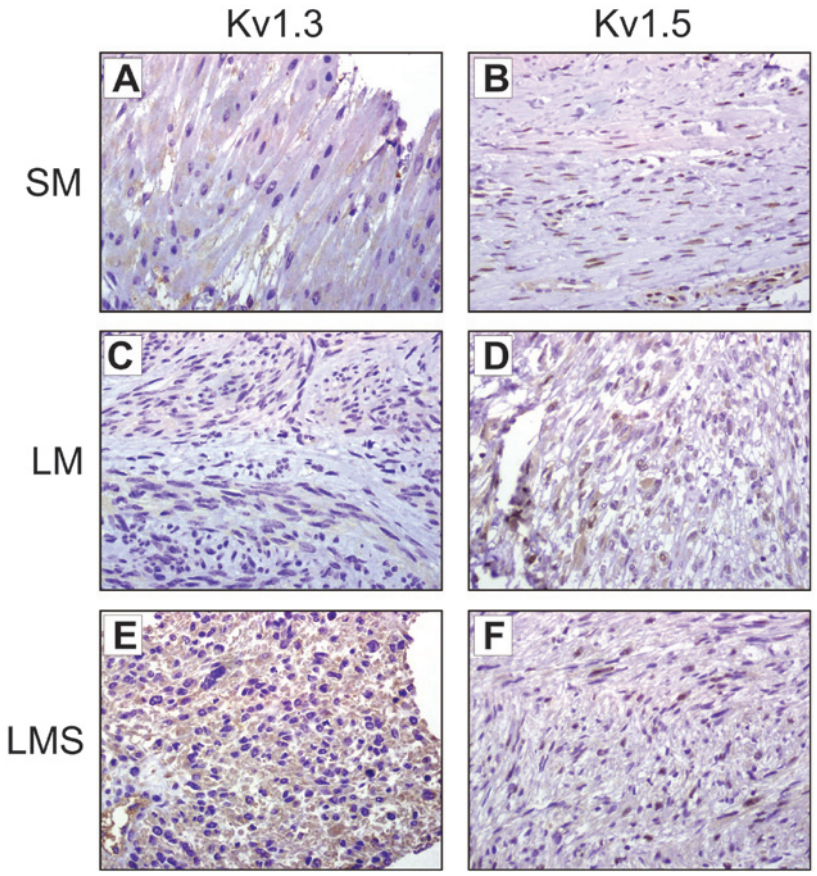

Figure 1. Representative images of Kv1.3 and Kv1.5 immunostaining in smooth muscle tumor and healthy samples. (A and B) SM, uterine smooth muscle control. (C and D) LM, leiomyoma. (E and F) LMS, leiomyosarcoma. (A, C and E) Kv1.3. (B, D and F) Kv1.5.

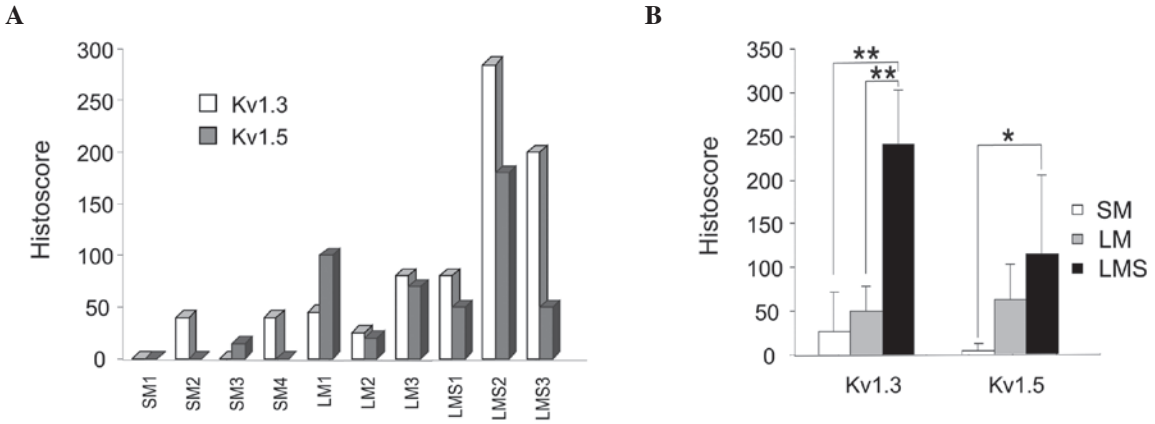

Figure 2. Graphical representation of the histoscore calculated from Kv1.3 and Kv1.5 immunohistochemistry. (A) Kv1.3 and Kv1.5 expression in smooth muscle tumor and control samples. SM1-SM4, smooth muscle control. LM1-LM3, leiomyoma samples. LMS1-LMS3, leiomyosarcoma samples. White bars, Kv1.3; gray bars, Kv1.5. (B) Histoscore mean values ± SEM for Kv1.3 and Kv1.5 in smooth muscle tumors and control samples. White bar, control smooth muscle tissue (SM); light gray bar, leiomyoma (LM); black bar, leiomyosarcoma (LMS). $^{*}p<0.05$ and $^{**}p<0.001$ (Student's t-test).

individual samples of smooth muscle tumors and healthy specimens. Fig. 2B shows the means ± SEM for different groups. Kv1.3 appeared to be the dominant channel in smooth muscle tumors. The expression of Kv1.3 in healthy smooth muscle was low or null, similar to that of the indolent LM, but its expression was notably increased in LMS (Fig. 2A and B). Kv1.3 expression in this aggressive sarcoma was 4-fold higher than that in the control samples (Fig. 2B), whereas Kv1.5 staining was slightly increased in the two types of tumors (Fig. 2A and B).

Thus, although the expression of the two channels showed a similar correlation with malignancy (Fig. 2B), the results were clear for Kv1.3. Kv1.3 staining was similar in the indolent LM and healthy specimens, but was significantly elevated in the aggressive LMS. A similar pattern was observed for Kv1.5, although to a lesser extent.

Discussion

Kv channels, which control cell excitability in muscle, also contribute to myoblast proliferation (2,11). In this study, we have shown for the first time that the expression of Kv1.3 and Kv1.5 increased in smooth muscle tumorigenesis in a close correlation with malignancy. Kv1.3, which governs smooth muscle proliferation and migration (2,3), increased notably in LMS. In addition, Kv1.5 expression, which steadily increases with aggressiveness, was shown to be almost absent in human myometrial smooth muscle.

Kv1.5 is involved in skeletal muscle cell proliferation (11). Unlike Kv1.5, Kv1.3 governs macrophage cell growth (14), but plays no substantial role in skeletal myoblast proliferation (11). In a recent study, we observed a correlation of Kv1.3 and Kv1.5 expression with tumor malignancy in rhabdomyosarcomas (16). However, Kv1.3 staining revealed no major differences between tumors and healthy samples (16). Therefore, the role of Kv1.3 in skeletal myoblasts is uncertain. By contrast, Kv1.3 action on smooth muscle proliferation seems defined and, similar to leukocytes, recent evidence supports the involvement of Kv1.3 in vascular smooth muscle cell (VMSC) proliferation and migration (2,3).

Kv1.3 and Kv1.5 remodel in a large variety of human cancers (13). In addition, their expression correlates with rhabdomyosarcoma aggressiveness (16). Much evidence supports a role for ion channels in cancer development, progression and metastasis, and Kv1.3 and Kv1.5 may serve as potential biomarkers and/or anti-cancer therapeutic targets (9). In our study, aggressive LMS exhibited Kv1.3 staining higher than that of indolent LM and control smooth muscle biopsies. In addition, Kv1.5 expression also increased in LMS. Both results are in agreement with previous reports demonstrating that Kv1.5 and Kv1.3 play a role in skeletal and smooth muscle cell proliferation (2,3,11).

VSMCs are responsible for the correct contraction and dilatation of blood vessels, which play a crucial role in hypertension. In addition, VSMCs are capable of changing their phenotype from contractile to proliferative (2,3). This is an important feature during wound healing, but it may also become pathological in neointimal hyperplasia. The expression pattern of Kv undergoes a marked change during this switch. While contractile murine VSMCs express almost all isoforms of the Kv1 family (Kv1.1 - Kv1.6), which control the vascular tone (3,20-22), proliferating cells lose the expression of the majority of the VSMCs, solely upregulating Kv1.3 (2,3). Thus, proliferating human vein smooth muscle cells undergoing intimal neoplasia express high levels of Kv1.3, which further supports a putative role in VSMC proliferation. In this situation, Kv1.3 blockers inhibit growth and migration of venous cells (2,3). In this context, our results support findings of previous reports, suggesting Kv1.3 as a potential target for pathologies involving the excessive proliferation of smooth muscle cells (2,3).

In conclusion, these results demonstrate for the first time that Kv1.3 and Kv1.5 are specifically remodeled during smooth muscle carcinogenesis. A notable Kv1.3 expression is associated with smooth muscle cancer aggressiveness and a correlation of Kv1.5 expression is observed with tumorigenesis. Furthermore, our study argues in favor of Kv channels as potential therapeutic targets in pathologies involving excessive cell proliferation.

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References

1. Hille B: Ion Channels of Excitable Membranes. 3rd edition. Sinauer, Sunderland, MA, xviii, 814, 2001.
2. Cidad P, Moreno-Dominguez A, Novensa L, *et al*: Characterization of ion channels involved in the proliferative response of femoral artery smooth muscle cells. Arterioscler Thromb Vasc Biol 30: 1203-1211, 2010.
3. Cheong A, Li J, Sukumar P, *et al*: Potent suppression of vascular smooth muscle cell migration and human neointimal hyperplasia by KV1.3 channel blockers. Cardiovasc Res 89: 282-289, 2011.
4. Conti M: Targeting K^+ channels for cancer therapy. J Exp Ther Oncol 4: 161-166, 2004.
5. Felipe A, Vicente R, Villalonga N, *et al*: Potassium channels: new targets in cancer therapy. Cancer Detect Prev 30: 375-385, 2006.
6. Kunzelmann K: Ion channels and cancer. J Membr Biol 205: 159-173, 2005.
7. Pardo LA: Voltage-gated potassium channels in cell proliferation. Physiology (Bethesda) 19: 285-292, 2004.
8. Wonderlin WF and Strobl JS: Potassium channels, proliferation and G1 progression. J Membr Biol 154: 91-107, 1996.
9. Felipe A, Bielanska J, Comes N, *et al*: Targeting the voltage-dependent K^+ channels Kv1.3 and Kv1.5 as tumor biomarkers for cancer detection and prevention. Curr Med Chem 9: 661-674, 2011.
10. Vicente R, Escalada A, Villalonga N, *et al*: Association of Kv1.5 and Kv1.3 contributes to the major voltage-dependent K^+ channel in macrophages. J Biol Chem 281: 37675-37685, 2006.
11. Villalonga N, Martinez-Marmol R, Roura-Ferrer M, *et al*: Cell cycle-dependent expression of Kv1.5 is involved in myoblast proliferation. Biochim Biophys Acta 1783: 728-736, 2008.
12. Swanson R, Marshall J, Smith JS, *et al*: Cloning and expression of cDNA and genomic clones encoding three delayed rectifier potassium channels in rat brain. Neuron 4: 929-939, 1990.
13. Bielanska J, Hernandez-Losa J, Perez-Verdaguer M, *et al*: Voltage-dependent potassium channels Kv1.3 and Kv1.5 in human cancer. Curr Cancer Drug Targets 9: 904-914, 2009.
14. Vicente R, Escalada A, Coma M, *et al*: Differential voltage-dependent K^+ channel responses during proliferation and activation in macrophages. J Biol Chem 278: 46307-46320, 2003.
15. Villalonga N, Escalada A, Vicente R, *et al*: Kv1.3/Kv1.5 heteromeric channels compromise pharmacological responses in macrophages. Biochem Biophys Res Commun 352: 913-918, 2007.
16. Bielanska J, Hernandez-Losa J, Moline T, *et al*: Differential expression of Kv1.3 and Kv1.5 voltage-dependent K^+ channels in human skeletal muscle sarcomas. Cancer Invest 30: 203-208, 2012.
17. Bielanska J, Hernandez-Losa J, Moline T, *et al*: Voltage-dependent potassium channels Kv1.3 and Kv1.5 in human fetus. Cell Physiol Biochem 26: 219-226, 2010.
18. Lan M, Shi Y, Han Z, *et al*: Expression of delayed rectifier potassium channels and their possible roles in proliferation of human gastric cancer cells. Cancer Biol Ther 4: 1342-1347, 2005.
19. Castellvi J, Garcia A, Ruiz-Marcellan C, *et al*: Cell signaling in endometrial carcinoma: phosphorylated 4E-binding protein-1 expression in endometrial cancer correlates with aggressive tumors and prognosis. Hum Pathol 40: 1418-1426, 2009.
20. Archer SL, Souil E, Dinh-Xuan AT, *et al*: Molecular identification of the role of voltage-gated K^+ channels, Kv1.5 and Kv2.1, in hypoxic pulmonary vasoconstriction and control of resting membrane potential in rat pulmonary artery myocytes. J Clin Invest 101: 2319-2330, 1998.
21. Chen TT, Luykenaar KD, Walsh EJ, Walsh MP and Cole WC: Key role of Kv1 channels in vasoregulation. Circ Res 99: 53-60, 2006.
22. Nelson MT and Quayle JM: Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 268: C799-C822, 1995.