Am J Clin Exp Urol 2019;7(3):178-181
www.ajceu.us /ISSN:2330-1910/AJCEU0096298

Original Article

KIF3B protein expression loss correlates with metastatic ability of prostate cancer

Oleksandr Kravtsov[1], Christopher P Hartley[1], Eva-Maria Compérat[2], Kenneth A Iczkowski[1]

[1]Department of Pathology, Medical College of Wisconsin, Milwaukee, WI, USA; [2]Hôpital Tenon, Assistance Publique-Hôpitaux de Paris, Université Pierre et Marie Curie, Paris, France

Received April 29, 2019; Accepted May 30, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: Kinesin family member 3B (KIF3B) is a microtubule motor kinesin involved in mitotic progression and vasculotropism. A novel therapeutic target, it is overexpressed in several cancers [PMID 29904055]. Its significance in prostate cancer (PC) was uncertain. Methods: 89 cases, including tissue microarrays from 70 prostatectomies comprising matched cancer and benign spots, 19 additional prostatectomy tissues, plus 16 prostate cancer metastases (7 nodal and 9 distant sites; 8 had matched primary PC) were stained with rabbit polyclonal KIF3B antibody. Cytoplasmic immunoreactivity was scored: 0 (negative) to 3+ (strong and diffuse). 39 patients had no nodal metastases, 31 had positive lymph nodes, and 19 had nodes not sampled. Gleason grade groups were 1 (9), 2 (28), 3 (39), 4 (1), and 5 (12). 15 cases had cribriform pattern. AJCC stages were 2 (48), 3 (29), unknown (12). Results: KIF3B in PC (mean 1.0) was higher than in benign prostate (mean 0.1, P<0.01, Student t-test). All 7 available nodal metastases of PC were negative. One-third of primary PCs with nodal metastases lost all expression, compared to retained expression in all but one PC without nodal metastasis (P<0.01, chi-square). The former group also had stronger staining (mean 1.0) than metastases (mean 0.3) (P<0.01, Student t-test) and had fewer cases with any positive (>0) expression compared to cases without metastases or with unsampled lymph nodes (P<0.01, chi-square test). Reactivity of paired metastatic tissue and primary PC correlated strongly (Pearson coefficient: +0.7). No significant trends were found by grade group, cribriform status, or stage. Conclusions: KIF3B is a PC marker. Metastatic cancers showed less KIF3B expression than their primary PC counterparts, and primary cases with positive nodes demonstrated reduced positivity, suggesting use as a prognostic marker. It is possible that KIF3B protein becomes altered prior to metastases, preventing immunohistochemical detection.

Keywords: Microtubule, KIF3B, prostate cancer, metastasis

Introduction

Kinesin family member 3B (KIF3B) is a microtubule motor kinesin, involved in regulating intracellular transport, spermatogenesis, mitotic progression, and intravasation of cancer cells for metastasis [1]. A recent quantitative in vivo whole genome motility screen revealed KIF3B as a top therapeutic target [1]. KIF3B is overexpressed in different types of cancer, including acute lymphoblastic leukemia, seminoma, oral squamous cell, prostate, and hepatocellular carcinoma [1-5]. Its inhibition by shRNA in an avian embryo model most strongly inhibited prostate cancer PC3 and other cancer cell vasculotropism and metastasis [1]. Increased expression of KIF3B was correlated with poor survival in patients with hepatocellular carcinoma and its inhibition decreased cancer growth and induced tumor apoptosis [5]. Downregulation of another member of kinesin superfamily proteins, KIF23, has been suggested to decrease glioma development [6]. Thus, KIF3B is a novel therapeutic target to block cancer metastasis and inhibit cancer development.

To our knowledge, the clinical significance of KIF3B expression has not been studied in primary and metastatic prostate cancer (PC). The following study examines KIF3B protein expression in primary and metastatic PC with respect to benign versus cancer epithelium, tumor grade, the presence of a cribriform pattern (an adverse prognostic finding that exerts an influence independent of numerical Gleason score, reviewed [7]), tumor stage, primary can-

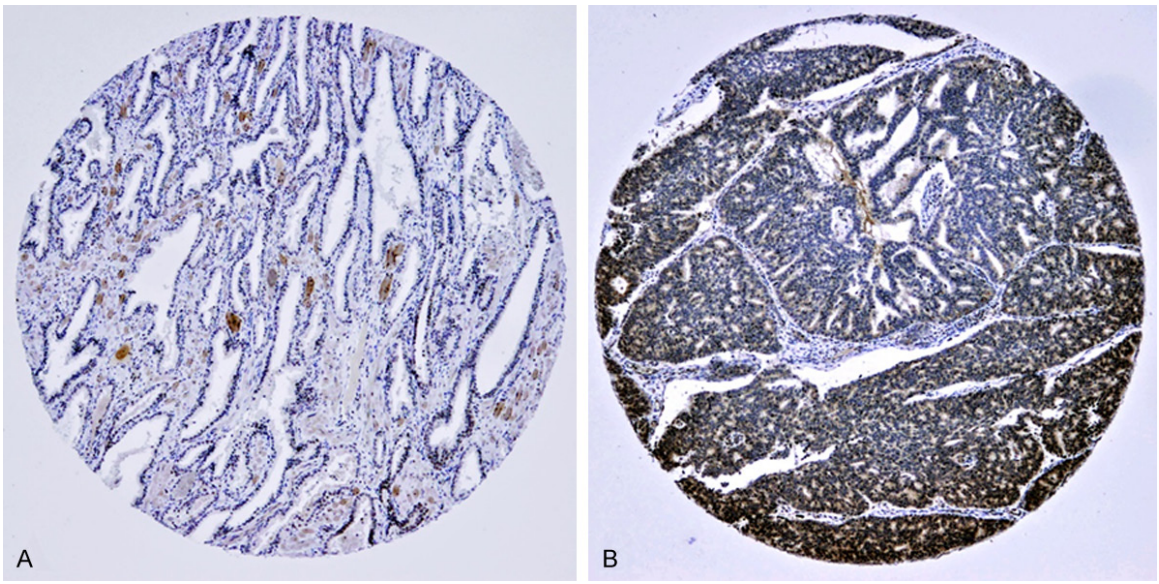

Figure 1. A. KIF3B immunoexpression in benign prostate. B. Typical KIF3B immunoexpression in cancer.

cer with metastases versus without, and metastatic cancer.

Materials and methods

Clinical characteristics and immunostaining

Tissue microarrays from 70 prostatectomies and unstained slides from 19 additional prostatectomy cases and 15 PC metastases (8 distant and 7 nodal) were stained with KIF3B antibody. Each TMA case contained 3-4 punch cores of PC and 3-4 of matched benign prostate. Antigen retrieval was done in Tris buffer at pH 9 for 20 minutes at 97 degrees Celsius. Rabbit polyclonal KIF3B antibody clone orb18-4776 (Biorbyt, United Kingdom) was used at 1:400 dilution on Dako platform. Two pathologists independently scored cytoplasmic immunoreactivity (**Figure 1**) from 0 (negative) to 3+ (strong and diffuse), including half-steps. Major discrepancies were resolved by consensus.

Lymph node status was obtained from pathology reports on all 89 prostatectomy cases. 39 patients had no nodal metastases, 31 had positive lymph nodes, or nodes were not sampled at prostatectomy (19). Distant sites of metastasis were following: liver-4 cases, bone-2 cases, brain-1 case, and spinal cord-1 case. Matched primary PC was evaluated for KIF3B in 8 of 15 metastatic cases. Prostatectomy cases included Gleason grade group 1 (9), group 2 (28), group 3 (39), group 4 (1), and group 5 (12). Cribriform pattern was present in 15 cases and absent in 74. AJCC stage 2 PC (48), stage 3 (29) and unknown (12) were represented.

Statistical analysis

Student t-test was used to compare KIF3B stain values in PC (89 cases) and benign prostate (70 cases on tissue microarray only) and to analyze the differences in KIF3B stain values in lymph node-positive primary PC cases and stain values in metastatic tissue. Fisher exact test was used to assess differences by Gleason grade group. A Mann-Whitney U test was used to compare KIF3B stain values in PC by cribriform status and by AJCC stage. A chi-square test was used to compare numbers of PC cases with any KIF3B stain positivity in tumor (stain value >0) and numbers of PC cases with negative KIF3B staining among the PC group with lymph node metastases and PC with no metastases, and PC group with lymph node metastases and PC with no metastases plus PC cases with unsampled lymph nodes. Fisher exact test was used to compare numbers of PC cases with KIF3B stain value >1 and numbers of PC cases with absent KIF3B staining by Gleason grade groups. Only 1 case of Gleason grade group 4 (total score of 8) was identified in the set and was omitted from grade comparisons. Pearson correlation coefficient was obtained to assess associations between KIF3B stain values of paired metastatic tissue and primary PC.

Table 1. KIF3B immunoexpression in PC, benign prostate, PC with lymph node metastases, metastatic tissue, and PC stratified by cribriform status and AJCC stage

	Mean stain value	±SD	N (% from total PC)	*p*-value
PC (all)	1.0	0.7	89 (100)	<0.01
Benign prostate	0.1	0.2	70	
PC with LN metastases	1.0	1.0	31 (35)	<0.01
Metastatic tissue (7 LN and 8 distant metastases)	0.3	0.7	15	
Cribriform PC	1.4	1.0	15 (17)	0.13
Non-cribriform PC	0.9	0.7	74 (83)	
AJCC stage 2 PC	1.0	0.6	48 (62)	0.13
AJCC stage 3 PC	1.1	0.9	29 (38)	

PC = prostate cancer, SD = standard deviation, LN = lymph node. Stain values in PC versus benign prostate and PC with LN metastases versus metastatic tissue compared using Student t-test (both comparisons yielded *p*-value <0.01). Mann-Whitney U test was used to assess the KIF3B stain differences in PC stratified by cribriform status and by AJCC stage (both comparisons yielded *p*-value 0.13).

Table 2. KIF3B staining by Gleason grade group

	Number of cases, total = 88	KIF3B stain value >1, N	*p*-value
Gleason grade Group 1 (3+3)	9	3 (33%)	0.67
Gleason grade Group 2 (3+4)	28	11 (39%)	
Gleason grade Group 3 (4+3)	39	17 (44%)	
Gleason grade Group 5 (total score of 9 or 10)	12	7 (58%)	

PC = prostate cancer. Fisher exact test was used to compare number of PC cases with KIF3B stain value of >1 in Gleason groups 1, 2, 3 and 5. Only 1 case of group 4 was present in the study and was excluded from the comparisons.

Results

KIF3B stain values in PC (mean: 1.0) were significantly higher than benign prostate (mean: 0.1, **Table 1**; **Figure 1**; Supplemental Data). None of the seventy cases of benign prostate had a stain value of more than 1 (median value: 0). There were no significant differences noted by cribriform status, AJCC stage (**Table 1**), or grade group (**Table 2**).

KIF3B stain values in primary PC with lymph node metastases (mean: 1.0) were higher than the stain values in metastatic tissue (mean: 0.3, P<0.01, **Table 1**). A strong positive correlation (Pearson correlation coefficient: +0.7) was noted in KIF3B stain values of paired metastatic tissues and primary PC. All 7 nodal metastases of PC had negative KIF3B expression.

Finally, one-third (11 of 31) of primary PCs that had lymph nodal metastasis were negative for KIF3B expression. This differed significantly in an independent comparison to 39 PCs without lymph node metastases, of which all but one were positive. When PCs from operations in which nodes were not sampled (probably overwhelmingly negative) were added to those with negative nodes, the comparison to primary cancers with positive nodes was also significant (P< 0.01, **Table 3**).

Discussion

KIF3B enables cancer cell motility and has been proposed as a therapeutic target in cancer [1]. It is a direct target of miR-127-3p [4] and miR-127 [8], so microRNAs can be used to counteract its effects. We have found that KIF3B protein was greatly elevated in prostate cancer (PC). In fact, when strongly positive, KIF3B immunohistochemical stain may specifically distinguish PC from benign prostate, since none of our benign prostate tissues stained strongly for KIF3B (mean value: 0.3, median value: 0). Significantly higher KIF3B expression in PC compared to benign prostate suggests that KIF3B may be involved in PC cancer progression in a fashion similar to hepatocellular carcinoma and other cancer types [5].

Not only did metastatic tissue show less KIF3B expression than the primary prostate cancer counterparts, but also there were fewer KIF3B positive primary PCs among those with positive lymph nodes (P<0.01). This finding suggests KIF3B may serve as a prognostic marker in PC. It stands in contrast to other tumor models; for example RNAi-mediated inhibition of KIF3B

Table 3. KIF3B staining by lymph node status

	Any positive KIF3B staining	Number of cases, total = 89	*p*-value
PC with lymph node metastases (31 cases)	Yes	20 (23%)	<0.01
	No	11 (12%)	
PC without metastases (39 cases)	Yes	38 (43%)	<0.01
	No	1 (1%)	
PC without metastases + cases in which lymph nodes were unsampled (58 cases)	Yes	55 (62%)	<0.01
	No	3 (3%)	

PC = prostate cancer, LN = lymph node. Chi-square test was used to compare numbers of PC cases with any KIF3B stain positivity in tumor (stain value >0) and numbers of PC cases with negative KIF3B staining among the PC group with LN metastases and PC with no metastases, and PC group with LN metastases and PC with no metastases plus PC cases with unsampled lymph nodes (both group comparisons yielded *p*-value <0.01).

blocked metastasis of head/neck HEp3 tumor cells in mice, and in vitro migration of prostate cancer PC3 cells [1].

One limitation of our study is the use of only an immunohistochemical method of KIF3B detection. It is possible that KIF3B protein remains overexpressed in metastases, but undergoes a mutation or other alteration, making it undetectable by conventional immunohistochemistry. In situ hybridization or other assessment of RNA transcription would answer this question.

Conclusion

KIF3B, a microtubule motor kinesin, was not unexpectedly increased in prostate cancer (PC) compared to benign epithelium. The loss of KIF3B immunoexpression in terms of staining strength and number of positive cases in primary PC with metastases, and in nodal and distant metastatic tumor, suggests either a mutation that impedes immunodetection or true downregulation. Further work should determine whether metastasis involves KIF3B protein that is overexpressed but is mutated, or whether loss of expression actually precedes metastasis.

Acknowledgements

The funding for this work was internal departmental funding.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kenneth A Iczkowski, Department of Pathology, Medical College of Wisconsin, 9200 W. Wisconsin Ave., Milwaukee, WI 53226, USA. E-mail: kaiczkowski@mcw.edu

References

[1] Stoletov K, Willetts L, Paproski RJ, Bond DJ, Raha S, Jovel J, Adam B, Robertson AE, Wong F, Woolner E, Sosnowski DL, Bismar TA, Wong GK, Zijlstra A, Lewis JD. Quantitative in vivo whole genome motility screen reveals novel therapeutic targets to block cancer metastasis. Nat Commun 2018; 9: 2343.

[2] An Q, Wright SL, Moorman AV, Parker H, Griffiths M, Ross FM, Davies T, Harrison CJ, Strefford JC. Heterogeneous breakpoints in patients with acute lymphoblastic leukemia and the dic(9;20)(p11 13;q11) show recurrent involvement of genes at 20q11.21. Haematologica 2009; 94: 1164-1169.

[3] Shen HQ, Xiao YX, She ZY, Tan FQ, Yang WX. A novel role of KIF3b in the seminoma cell cycle. Exp Cell Res 2017; 352: 95-103.

[4] Ji L, Zhu ZN, He CJ, Shen X. MiR-127-3p targets KIF3B to inhibit the development of oral squamous cell carcinoma. Eur Rev Med Pharmacol Sci 2019; 23: 630-640.

[5] Huang X, Liu F, Zhu C, Cai J, Wang H, Wang X, He S, Liu C, Yao L, Ding Z, Zhang Y, Zhang T. Suppression of KIF3B expression inhibits human hepatocellular carcinoma proliferation. Dig Dis Sci 2014; 59: 795-806.

[6] Takahashi S, Fusaki N, Ohta S, Iwahori Y, Iizuka Y, Inagawa K, Kawakami Y, Yoshida K, Toda M. Downregulation of KIF23 suppresses glioma proliferation. J Neurooncol 2012; 106: 519-529.

[7] Iczkowski KA, van der Kwast TA, Paner G. The new realization about cribriform prostate cancer. Adv Anat Pathol 2018; 25: 31-37.

[8] Shen Z, Jiang H, Hsu HT, Qian L, Fu Q, Shen M, Chen S, Yang T. MicroRNA-127 inhibits cell proliferation via targeting Kif3b in pancreatic β cells. Aging (Albany NY) 2019; 11: 1342-1355.