Clinical Study

Chemotherapy

Chemotherapy 2009;55:407–413
DOI: 10.1159/000263227

Received: March 17, 2009
Accepted after revision: June 24, 2009
Published online: December 2, 2009

Expression of FXYD3 Protein in Relation to Biological and Clinicopathological Variables in Colorectal Cancers

Emma Widegren[a] Sofia Önnesjö[a] Gunnar Arbman[b] Hany Kayed[d]
Hanswalter Zentgraf[e] Jörg Kleeff[f] Hong Zhang[c] Xiao-Feng Sun[a]

[a]Departments of Oncology, Institute of Clinical and Experimental Medicine, Linköping University, Linköping, [b]Department of Surgery, Vrinnevi University Hospital, Norrköping, and [c]School of Life Sciences, University of Skövde, Skövde, Sweden; [d]Institute of Clinical Radiology and Nuclear Medicine, University Hospital Mannheim, University of Heidelberg, and [e]Applied Tumour Virology, German Cancer Research Centre, Heidelberg, and [f]Department of Surgery, Technische Universität München, Munich, Germany

Key Words
Colorectal cancer · FXYD3 · Immunohistochemistry

Abstract
Background: FXYD3 is up-/down-regulated in different types of cancers. We examined FXYD3 expression in colorectal cancers and its relationship to biological and clinicopathological variables. ***Patients and Methods:*** Expression of FXYD3 protein was immunohistochemically examined in distant normal mucosa (n = 34), adjacent normal mucosa (n = 72), primary tumour (n = 150) and lymph node metastasis (n = 35) from colorectal cancer patients. ***Results:*** FXYD3 was highly expressed in primary tumour compared to adjacent normal mucosa (p = 0.02). FXYD3 was or tended to be positively related to the expression of ras (p = 0.02), p53 (p = 0.06), legumain (p = 0.02) and proliferating cell nuclear antigen (p = 0.03). Moreover, there was a higher frequency of strong FXYD3 expression in Dukes A–C tumours than in D tumours (p = 0.04). The strong FXYD3 expression tended to predict worse survival in the patients with Dukes A + B tumour (p = 0.07), while there was no such tendency in the patients with Dukes C + D tumour (p = 0.94). The tumours located in the colon had a higher degree of FXYD3 expression than the tumours located in the rectum (p = 0.05). ***Conclusion:*** The FXYD3 was associated with certain biological variables and may be involved in the development of the relative earlier stages of colorectal cancers.

Copyright © 2009 S. Karger AG, Basel

Background

FXYD3 (8 kDa), also known as mammary tumour (Mat)-8, is a member of the FXYD protein family that comprises 7 members [1, 2]. FXYD3 has been proven to be located on both surface and intracellular membranes in cells including colon cancer cells and to interact with Na-K-ATPase as a chloride channel or a chloride channel regulator [1, 3, 4]. FXYD3 differs from the other family members as, for instance, it has 2 transmembrane segments [1].

In normal tissues, FXYD3 is mainly expressed in the colon, stomach, uterus and skin. In the tumours, FXYD3 expression varies, and it can be up-regulated or down-regulated in the different types of tumours [1, 2, 4, 5]. For example, FXYD3 is over-expressed in pancreatic tumour, breast cancer and androgen-dependent prostate cancer,

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2009 S. Karger AG, Basel
0009–3157/09/0556–0407$26.00/0

Accessible online at:
www.karger.com/che

Prof. Xiao-Feng Sun, MD, PhD
Department of Oncology, Institute of Clinical and Experimental Medicine
Linköping University
SE–581 85 Linköping (Sweden)
Tel. +46 1322 2066, Fax +46 1322 3990, E-Mail xiao-feng.sun@liu.se

whereas it seems to be down-regulated in androgen-independent prostate cancer [5, 6].

FXYD3 has been postulated to have both proliferative and anti-proliferative effects on cells. Suppression of FXYD3 protein with siRNA in prostate carcinoma cell lines results in a declined proliferation, although the cells show the same invasive capacity and apoptosis rate. The data indicate that FXYD3 is involved in the growth of the prostate tumours and exerts proliferative effects [7]. On the other hand, some studies have shown that FXYD3 exerts an anti-proliferative action in breast cancer cells, or no effect on proliferation in colon cancer cells [8, 9].

FXYD3 promoter contains the binding sites for p53, indicating that the p53 might be involved in the regulation of FXYD3 expression. A study has shown that FXYD3 expression is dramatically increased after fluoropyrimidine 5-fluorouracil exposure only in the cell line with a functional p53, while FXYD3 expression is unchanged in a p53-null cell line. These findings provide evidence that p53 is, at least in part, associated with FXYD3 expression [8]. The fact that FXYD3 is up-regulated in a colon cancer cell line treated with 5-fluorouracil may indicate that the FXYD3 protein is implicated in the chemo-response/resistance of the colon cancer cells.

The aim of our study was to examine the expression of FXYD3 protein in distant normal mucosa, adjacent normal mucosa, primary tumour and lymph node metastasis from colorectal cancer patients, and the relationship of FXYD3 expression with biological and clinicopathological variables.

Patients and Methods

Patients

For immunohistochemistry, formalin-fixed, paraffin-embedded tissue samples were obtained from 150 colorectal cancer patients who underwent surgical resection at Linköping University Hospital, Linköping, and Vrinnevi University Hospital, Norrköping, Sweden, during the period of 1977–1997. This study included 34 distant normal colorectal mucosa specimens (32 of them were matched with primary tumours) taken from the margin of distant resection and 35 metastases (33 of them were matched with primary tumours) from the regional lymph nodes of the colon/rectum. Among the primary tumours, 72 tumours had adjacent normal mucosa, that is normal mucosa adjacent to the primary tumour. Both the distant and adjacent normal mucosa were histologically free from pre-tumorous and tumorous lesions. The patients' gender, age, tumour location and Dukes stage were obtained from surgical and/or pathological records at Linköping and Vrinnevi University Hospital. There were 83 men and 67 women, and the mean age was 71 years old (range 34–89). The growth patterns of tumours were based on the pattern of tumour growth and invasiveness. Differentiation of tumours was graded as better (good and moderate) and worse (poor and mucinous and signet-ring cell carcinoma). Inflammatory infiltration of tumours was scored as weak or strong infiltration. The patients were followed up until May 2006, and 67 patients died of colorectal cancer. Data of ras [10], p53 [11], proliferating cell nuclear antigen (PCNA) [12] and legumain expression [13] were taken from previous studies carried out at our laboratory. The variation of the case numbers of the biological variables (table 1) and the clinicopathological variables (table 2) are due to the number of available cases. The study was approved by the ethical committee at the Faculty of Health Sciences, University of Linköping, Sweden.

Immunohistochemical Staining

Five-micrometre thick formalin-fixed paraffin-embedded sections were used for immunohistochemical staining. The sections were incubated at 60°C for 12 h, deparaffinised and then rehydrated. For antigen retrieval, the sections were transferred to 0.01 M Tris-EDTA buffer (pH 9.0) and cooked in a high-pressure cooker for 8 min, and then incubated at room temperature for 30 min. After washing in PBS (pH 7.4), the sections were incubated with 3% H_2O_2 in methanol for 20 min to block endogenous peroxidase activity. The sections were pre-incubated with serum-free Dako Protein Block (Dako, Carpinteria, Calif., USA) for 10 min to block non-specific binding of antibody. After removing the blocking solution, the sections were incubated with a monoclonal anti-FXYD3 primary antibody [6] in 1:2 dilution with antibody diluent (Dako) overnight at 4°C in a moist chamber at room temperature. Subsequently, the sections were incubated with a goat anti-mouse/rabbit immunoglobulin, which is a component in ChemMate Dako Envision Detection Kit (DakoCytomation, Glostrup, Denmark) for 25 min. The sections were washed with PBS 3 times between each incubation step. Peroxidase reaction was performed for 8 min in 3,3-diaminobenzidine tetrahydrochloride (DAB) solution (DakoCytomation). The sections were rinsed with water and counterstained with Mayer's haematoxylin and then washed, dehydrated in ethanol and mounted with xylene-based mounting medium. Sections known to show strong immunostaining for FXYD3 were used in each run receiving either the primary antibody or universal mouse IgG (Dako) as positive and negative controls. In all the staining procedures, the positive controls showed clear staining and there was no staining in the negative controls.

The stained sections were microscopically examined and scored independently by 2 of the authors without any information of the biological and clinicopathological data. When the examiners provided scores that were not in agreement, the slides concerned were once again examined separately. Among the 219 sections from 150 cases evaluated, there was discrepancy in 25 sections in the first round of evaluation. These sections were re-read individually and matched. The final 11 discrepant sections were re-examined by dual-microscope and scored accordingly in agreement.

The expression of the FXYD3, if positive, was present in the cytoplasm of normal epithelial and tumour cells. The staining was scored as negative, weak, moderate and strong based on the intensity of the staining in the cytoplasm of normal epithelial and tumour cells. Considering the similarities of the clinicopathological features, the cases with negative and weak expressions of the FXYD3 were grouped as 'weakly stained' and the cases with mod-

erate and strong expression as 'strongly stained'. In order to avoid artefacts, tissue in the areas with poor morphology, necrosis and in the margins of the sections were not considered during slide reading.

Statistical Analysis

The significance of the difference in intensity of the FXYD3 expression between normal mucosa samples and primary tumours and metastases was tested by the χ^2 test or the McNemar's method. The relationships between the FXYD3 expression and biological or clinicopathological variables were tested by the χ^2 test. The relationship between the FXYD3 expression and survival was tested using Cox's Proportional Hazard Model. Survival curves were calculated by using the Kaplan-Meier method. Two-sided p values of less than 5% were considered as statistically significant.

Results

Expression of the FXYD3 Protein in Normal Mucosa, Primary Tumour and Lymph Node Metastasis

Expression of the FXYD3 protein was examined in normal mucosa, primary tumour and metastasis in the lymph nodes. FXYD3 staining was in the cytoplasm, and there was no nuclear staining. FXYD3 was expressed in all specimens of the distant normal mucosa (n = 34), including weak staining in 15 (44%) specimens and strong in 19 (56%) specimens. In adjacent normal mucosa specimens (n = 72), FXYD3 showed weak staining in 38 (53%) specimens and strong staining in 34 (47%) specimens. Of the primary tumours (n = 150), 65 (43%) tumours showed weak FXYD3 expression and 85 (57%) tumours had strong expression. Among the metastases (n = 35), 13 (37%) were weak and 22 (63%) displayed strong expression of the FXYD3. FXYD3 staining in distant normal mucosa is shown in figure 1a, primary tumour in figure 1b and metastasis in the lymph node in figure 1c.

We further compared the FXYD3 expression in distant normal mucosa, adjacent normal mucosa, primary tumour and metastasis in the matched cases (the samples from the same patients) by using McNemar test. The frequency of the strong FXYD3 expression was significantly higher in primary tumour compared to adjacent normal mucosa (p = 0.02; table 3). There was no significant difference between the distant normal mucosa and primary tumour (p = 1.00), as well as between the primary tumour and the metastasis (p = 1.00, data not shown).

We also compared the staining intensity in the inner parts of tumour with that in the invasive margin of the tumour, and there was no significant difference in either the primary or metastatic tumours.

Table 1. FXYD3 expression in primary tumour in relation to biological variables in colorectal cancer

Variable	n	FXYD3 expression		p value[a]
		weak	strong	
ras				0.02
Negative	30	20 (67)	10 (33)	
Positive	46	18 (39)	28 (61)	
p53				0.06
Negative	70	36 (55)	34 (45)	
Positive	13	3 (35)	10 (65)	
Legumain				0.02
Weak	66	38 (58)	28 (42)	
Strong	24	7 (30)	17 (70)	
PCNA				0.03
Weak	41	24 (59)	17 (41)	
Strong	42	15 (36)	27 (64)	

Figures in parentheses are percentages. [a] χ^2 test.

Table 2. FXYD3 expression in primary tumour in relation to clinicopathological variables

Variable	n	FXYD3 expression		p value[a]
		weak	strong	
Gender				0.74
Male	83	35 (42)	48 (58)	
Female	67	30 (45)	37 (55)	
Age, years				0.53
<70	62	25 (40)	37 (60)	
≥70	88	40 (45)	48 (55)	
Tumour location				0.05
Colon	88	32 (36)	56 (64)	
Rectum	59	31 (53)	28 (47)	
Dukes stage				0.13
A	18	7 (39)	11 (61)	0.06[b]
B	55	20 (36)	35 (64)	0.04[c]
C	42	20 (48)	22 (52)	
D	27	17 (63)	10 (37)	
Growth pattern				0.33
Expansive	74	34 (46)	40 (54)	
Infiltrative	71	27 (38)	44 (62)	
Differentiation				0.30
Better	103	43 (42)	60 (58)	
Worse	47	22 (47)	25 (53)	
Inflammatory infiltration				0.08
Weak	93	34 (37)	59 (63)	
Strong	19	11 (58)	8 (42)	

Figures in parentheses are percentages.
[a] χ^2 test. [b] Dukes A + B vs. C + D. [c] Dukes A + B + C vs. D.

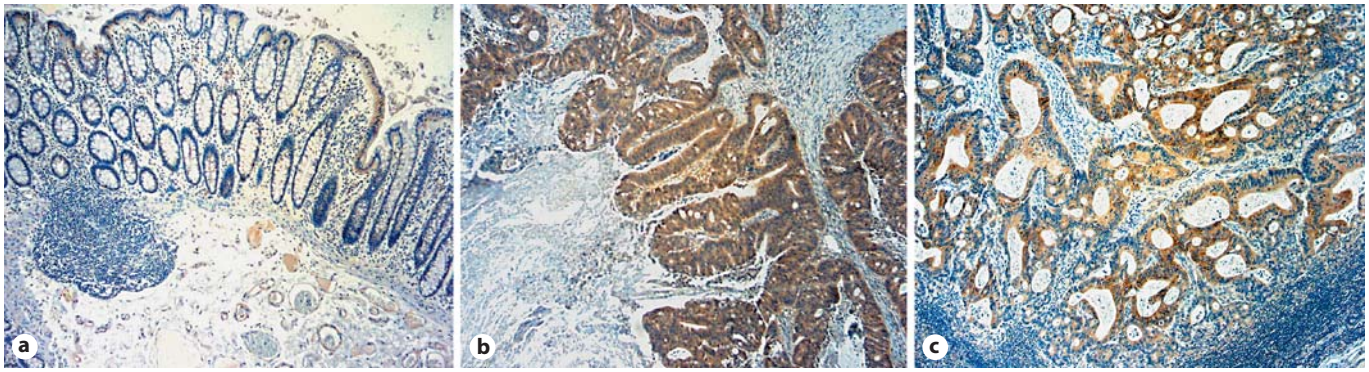

Fig. 1. Immunohistochemical assay for FXYD3 expression in colorectal cancer. FXYD3 had no expression in distant normal mucosa (**a**), and showed strong expression in primary tumour (**b**) and metastasis in the lymph node (**c**).

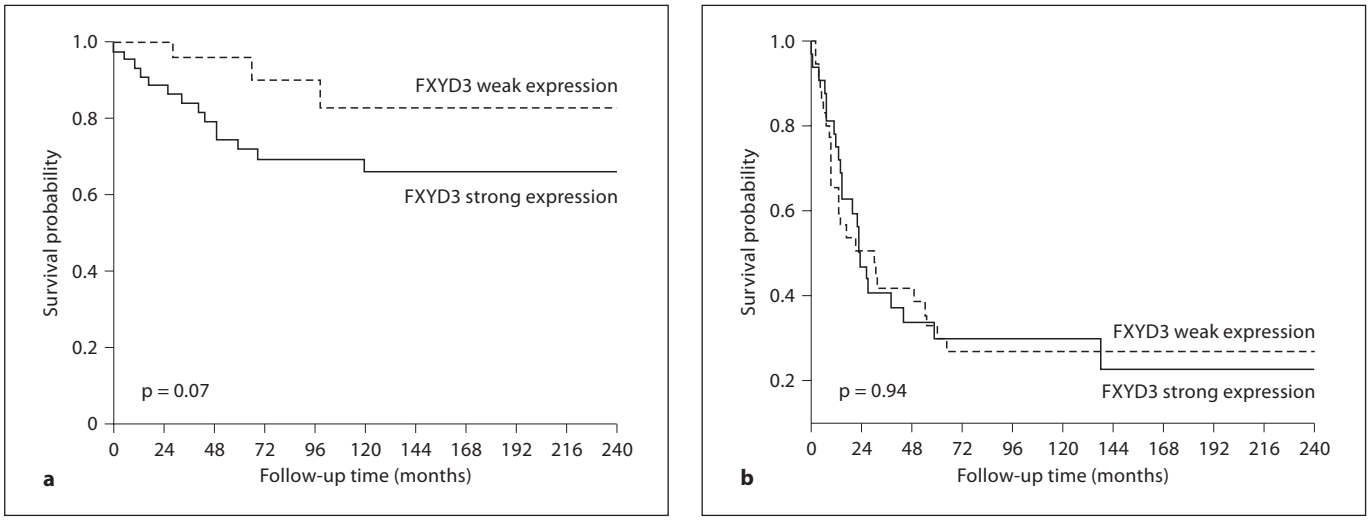

Fig. 2. FXYD3 expression in primary tumour in relation to survival of the patients with Dukes stage A + B (**a**) and Dukes stage C + D colorectal cancer (**b**).

Expression of the FXYD3 Protein in Primary Tumour in Relation to Biological and Clinicopathological Variables

Table 1 presents the relationship of FXYD3 expression with ras, p53, legumain and PCNA expression by using χ^2 test. FXYD3 expression was positively related to expression of ras ($p = 0.02$), legumain ($p = 0.02$) and PCNA ($p = 0.03$), and tended to be related to expression of p53 ($p = 0.06$).

Table 2 shows the relationships of FXYD3 expression in primary tumour cells with clinicopathological variables by using χ^2 test. There was a higher frequency of strong FXYD3 expression in colonic tumours (64%) than in rectal ones (47%, $p = 0.05$). FXYD3 expression did not have significant differences among individual Dukes stages ($p = 0.13$). However, when we compared the FXYD3 expression in the Dukes stage A–C tumours with that in the stage D tumours, the stage A–C tumours had a higher frequency of strong staining than did the stage D tumours (59 vs. 37%, $p = 0.04$). When stage A and B tumours were compared with stage C and D tumours, there was a tendency for the former to have a higher frequency of strong staining than the latter (63 vs. 47%, $p = 0.06$). There were no significant relationships of FXYD3 ex-

Table 3. Comparison of FXYD3 expression in adjacent normal mucosa with primary tumours

Adjacent normal mucosa	Primary tumour		Total	p value[a]
	weak	strong		
Weak	15 (21)	23 (32)	38 (53)	
Strong	9 (12)	25 (35)	34 (47)	
Total	24 (45)	48 (55)	72 (100)	0.02

Figures in parentheses are percentages. [a] For McNemar test.

pression with gender, age, growth pattern, differentiation and inflammatory infiltration ($p > 0.05$).

There was no correlation between the FXYD3 expression and survival in the whole group of the patients. We further analysed survival significance of the FXYD3 expression in the subgroups of the patients with Dukes stage A + B tumour (fig. 2a) and the patients with stage C + D tumour (fig. 2b). Strong expression of the FXYD3 tended to predict a worse prognosis than the weak expression in the patients with Dukes A + B tumour ($p = 0.07$), while there was no such tendency in the patients with Dukes C + D tumour ($p = 0.94$). There was no relationship between the FXFD3 expression and survival in the patients with Dukes stage A–C tumours, or with stage D tumours ($p > 0.05$).

Discussion

In the present study, we found that the frequency of the strong FXYD3 expression was significantly higher in primary tumour compared to adjacent normal mucosa in the matched cases, while there was no significant difference between primary tumour and metastasis in the lymph nodes. We also compared FXYD3 expression in inner parts of the tumour with that in the invasive margin of the primary and metastatic tumours, and did not find a significant difference. When we compared FXYD3 expression in Dukes stage A–C tumours with that in stage D tumours, we found that the former group had a higher frequency of the strong staining than the latter group. Although we did not see a significant relationship between the FXYD3 expression and survival in the patients with Dukes stage A–C tumours, strong FXYD3 expression in the patients with Dukes stage A + B tumour tended to have decreased survival, while there was no such relationship in the patients with C + D tumour. In our earlier study on rectal cancers, the FXYD3 expression in primary tumour was increased compared to either distant or adjacent normal mucosa, and had no significant difference from metastasis in the lymph nodes [14]. Other studies have shown that FXYD3 is over-expressed in the early process of malignant transformation in prostatic and pancreatic cancer [5, 6]. Taken together, these results indicate that there may be a change in FXYD3 expression during tumour development from normal epithelial cells to tumour cells, and that FXYD3 could be especially important in the development of tumours in the relative earlier stages.

There were controversial results regarding FXYD3 expression in normal cells versus tumour cells. By using RT-PCR analysis, Kayed et al. [5] found that colon cancers (n = 40) had down-regulated FXYD3 expression compared to normal colon mucosa samples (n = 27). Anderle et al. [15] compared FXYD3 expression in a colon cancer cell line, Caco-2, with that in human healthy colon (total RNA from a pool of 2 healthy male Caucasians, aged 35 and 65 years), and found by microarray that they had a similar level of the mRNA expression. In the present study, we did not see a significant difference in FXYD3 expression in primary tumours (n = 18) compared to distant normal colorectal mucosa (n = 14). While in another study of rectal cancer by our group that used the same method and antibody, the FXYD3 expression was over-expressed in primary tumour compared to either distant normal mucosa (n = 70) or adjacent normal mucosa (n = 101) [14]. We cannot state clear reasons for the different results between the FXYD3 expression in normal tissue and cancer tissue/cells; however, we propose that the results may depend on the methods for detecting FXYD3 expression, the number of cases, and sampling site (the colon, rectum, different clones within the same tumour, etc.). For example, in table 3, which shows FXYD3 expression in the matched cases (the samples from the same patients) of adjacent normal mucosa specimens and primary tumours, we saw that 23 adjacent normal mucosa specimens had the week FXYD3 expression but turned the strong expression in their matched primary tumours. However, we also saw 9 normal specimens that showed the strong FXYD3 expression, with weak FXYD3 expression in their matched primary tumours. By running a statistical analysis, we can conclude that there was a significant increase of FXYD3 expression in primary tumours compared with the matched normal mucosa ($p = 0.02$), but for the individual case, the FXYD3 expression in the normal versus primary tumours can be opposite or same.

In the present study, we compared the FXYD3 expression in tumour cells of colonic tumour with that rectal cancer, and found that colonic tumour tended to have a higher degree of FXYD3 expression than rectal tumours. Previous studies have shown that various proteins (e.g. stromelysin-3, matrix metalloproteinases, BCL2 and GSK3β) have different expressions in left- and right- sided colon tumours [16, 17]. Left-sided tumours display a worse prognosis than right-sided tumours in the patients with colorectal cancer [18]. Our present result indicates that the FXYD3 protein may be more involved in the development of colonic cancers than rectal cancers.

In the present study, we found that the FXYD3 expression was positively related to ras, p53, legumain and PCNA expression. Morrison and Leder [19] have shown that ras initiated murine mammary tumours leading to expression of FXYD3. In addition, the over-expression of ras correlates with a poor prognosis in colorectal cancer patients [10]. Mutated ras has been found in almost 40% of colorectal cancers, and correlates with an increased risk of metastasis and poor survival [18, 20]. p53, as a tumour suppressor gene, has an anti-proliferative and pro-apoptotic function [21]. Legumain is a cysteine endopeptidase that is correlated to migratory and invasive cells [13, 22]. An earlier study from our research group examining the legumain expression in some of the cases included in this study has shown that there is a significant increase of the legumain expression from adjacent normal colorectal mucosa to primary tumours, but there was no significant change between primary tumours and metastases in the lymph nodes. Over-expression of legumain was related to positive expression of p53 and PCNA as well as a poor prognosis [13]. Small interfering RNA-mediated inhibition of FXYD3 expression promotes a decrease of proliferation in prostate cancer [7]. Down-regulation of FXYD3 by stable antisense transfection increases the doubling time of pancreatic cancer cells, and, furthermore, mice transplanted with antisense-transfected cells significantly increase the doubling time of tumours [14]. Together with our present study showing a positive relationship of FXYD3 with PCNA, the data further strengthens the proliferative function of FXYD3.

Again, in Caco-2 cell line, silencing FXYD3 dose not affect cellular proliferation but promotes apoptosis and impeded differentiation [9]. This may depend on cell status, such as differentiation. FXYD3 may promote cellular proliferation in poorly-differentiated cancer cells, whereas FXYD3 has no effect on well-differentiated Caco-2 cells [9]. Morrison et al. [2] have examined FXYD3 expression in 15 different types of cells, and found that FXYD3 is not expressed by all cell types but only the colon, stomach, breast and uterus. In addition, FXYD3 may be initiated by ras and neu but not by c-MYC [19]. The results have indicated that FXYD3 does not perform a housekeeping function to all cells or that it plays its roles in an organ-, tissue- or cell-dependent manner and/or in different pathways. Thus, it is necessary to use more and different colon cancer cell lines, and even rectal cancer cell lines, to further identify the role of FXYD3 in colorectal cancers.

Conclusions

FXYD3 expression is related to several biological variables including ras, p53, legumain and PCNA, and may be involved in the development of the relatively early stages of colorectal cancers.

Acknowledgments

The authors are grateful to Helen Richard, Cecilia Bergenwald, Gertrud Stridh, Gunnel Lindell and Kerstin Ingels from the Department of Pathology, Linköping University, Sweden, for kindly preparing tissue sections. The study was supported by grants from the Swedish Cancer Foundation and the Health Research Council in the South-East of Sweden.

References

1 Geering K: FXYD3 proteins: new regulators of Na-K-ATPase. Am J Physiol Renal Physiol 2006;290:241–250.
2 Morrison BW, Moorman JR, Kowdley GC, Kobayashi YM, Jones LR, Leder P: Mat-8, a novel phospholemman-like protein expressed in human breast tumors, induces a chloride conductance in Xenopus oocytes. J Biol Chem 1995;270:2176–2182.
3 Crambert G, Li C, Claeys D, Geering K: FXYD3 (Mat-8), a new regulator of Na, K-ATPase. Mol Biol Cell 2005;16:2363–2371.
4 Arimochi J, Ohashi-Kobayashi A, Maeda M: Interaction of Mat-8 (FXYD-3) with Na+/K+-ATPase in colorectal cancer cells. Biol Pharm Bull 2007;30:648–654.
5 Kayed H, Kleeff J, Kolb A, Ketterer K, Keleg S, Felix K, Giese T, Penzel R, Zentgraf H, Büchler MW, Korc M, Friess H: FXYD3 is overexpressed in pancreatic ductal adenocarcinoma and influences pancreatic cancer cell growth. Int J Cancer 2006;118:43–54.

6 Vaarala MH, Porvari K, Kyllonen A, Vihko P: Differently expressed genes in two LNCaP prostate cancer cell lines reflecting changes during prostate cancer progression. Lab Invest 2000;80:1259–1268.
7 Grzmil M, Voight S, Thelen P, Hemmerlein B, Helmke K, Burfeind P: Up-regulated expression of the Mat-8 gene in prostate cancer and its siRNA-mediated inhibition of expression induces a decrease in proliferation of human prostate carcinoma cells. Int J Oncol 2004;29:97–105.
8 Maxwell PJ, Longley DB, Latif T, Boyer J, Allen W, Lynch M, McDermott U, Harkin DP, Allegra CJ, Johnston PG: Identification of 5-fluorouracil-inducible target genes using cDNA microarray profiling. Cancer Res 2003;63:4602–4606.
9 Bibert S, Aebischer D, Desgranges F, Roy S, Schaer D, Kharoubi-Hess S, Horisberger JD, Geering K: A link between FXYD3 (Mat-8)-mediated Na,K-ATPase regulation and differentiation of Caco-2 intestinal epithelial cells. Mol Biol Cell 2009;20:1132–1140.
10 Sun XF, Zhang H, Ekberg H, Carstensen JM, Nordenskjöld B: Overexpression of ras is an independent prognostic factor in colorectal adenocarcinoma. APMIS 1998;106:654–656.
11 Sun XF, Carstensen JM, Zhang H, Arbman G, Nordenskjöld B: Prognostic significance of p53 nuclear and cytoplasmic overexpression in right and left colorectal adenocarcinomas. Eur J Cancer 1996;11:1963–1967.
12 Sun XF, Carstensen JM, Stål O, Zhang H, Arbman G, Nordenskjöld B: Proliferating cell nuclear antigen (PCNA) in relation to ras, c-erbB-2, p53, clinico-pathological variables and prognosis in colorectal adenocarcinoma. Int J Cancer 1996;69:5–8.
13 Murthy R.V, Arbman G, Gao J, Roodman GD, Sun XF: Legumain expression in relation to clinicopathological and biological variables. Clin Cancer Res 2005;11:2293–2299.
14 Loftås P, Önnesjö S, Widgren E, Adell G, Kayed H, Kleef J, Zentgraf H, Sun XF: Expression of FXYD-3 is an independent prognostic factor in rectal cancer patients with preoperative radiotherapy. Int J Radiat Oncol Biol Phys 2009, E-pub ahead of print.
15 Anderle P, Rakhmanova V, Woodford K, Zerangue N, Sadée W: Messenger RNA expression of transporter and ion channel genes in undifferentiated and differentiated Caco-2 cells compared to human intestines. Pharm Res 2003;20:3–15.
16 Skoglund J, Emterling A, Arbman G, Anglard P, Sun XF: Clinicopathological significance of stromelysin-3 expression in colorectal cancer. Oncology 2004;67:67–72.
17 Komuro K, Tada M, Tamoto E, Kawakami A, Matsunaga A, Teramoto K, Shindoh G, Takada M, Murakawa K, Kanai M, Kobaya-shi N, Fujiwara Y, Nishimura N, Hamada J, Ishizu A, Ikeda H, Kondo S, Katoh H, Mo-riuchi T, Yoshiki T: Right- and left-sided colorectal cancers display distinct expression profiles and the anatomical stratification allows a high accuracy prediction of lymph node metastasis. J Surg Res 2005;124:216–224.
18 Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B: Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet 2000;355:1745–1750.
19 Morrison BW, Leder P: neu and ras initiate murine mammary tumours that share genetic markers generally absent in c-myc and int-2-initiated tumours. Oncogene 1994;9:3417–3426.
20 Andreyev HJN, Norman AR, Cunningham D, Oates JR, Clarke PA: Kirsten ras mutations in patients with colorectal cancer: the multicenter 'Rascal' study. J Natl Cancer Inst 1998;90:675–683.
21 Morgunkova AA: The p53 gene family: control of cell proliferation and developmental programs. Biochemistry 2005;70:955–971.
22 Liu C, Sun C, Huang H, Janda K, Edington T: Overexpression of Legumain in tumours is significant for invasion/metastasis and a candidate enzymatic target for prodrug therapy. Cancer Res 2003;63:2957–2964.