

SHORT COMMUNICATION

Fatty acid-binding protein 5 (FABP5)-related signal transduction pathway in castration-resistant prostate cancer cells: a potential therapeutic target

Abdulghani A. Naeem^{1,§}, Saud A. Abdulsamad^{1,§}, Philip S. Rudland², Mohammed I. Malki³ and Youqiang Ke^{1,*}

¹The Molecular Pathology Laboratory, Department of Molecular and Clinical Cancer Medicine; ²Department of Biochemistry, Liverpool University, Liverpool L69 3GA, UK; and ³College of Medicine, Qatar University, Doha 2713, Qatar

*Correspondence: Youqiang Ke, yqk@liverpool.ac.uk

Abstract

In this short communication, a novel fatty acid-binding protein 5 (FABP5)-related signal transduction pathway in prostate cancer is reviewed. In castration-resistant prostate cancer (CRPC) cells, the FABP5-related signal transduction pathway plays an important role during transformation of the cancer cells from androgen-dependent state to androgen-independent state. The detailed route of this signal transduction pathway can be described as follows: when FABP5 expression is increased as the increasing malignancy, excessive amounts of fatty acids from intra- and extra-cellular sources are transported into the nucleus of the cancer cells where they act as signalling molecules to stimulate their nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR γ). The phosphorylated or biologically activated PPAR γ then modulates the expression of its downstream target regulatory genes to trigger a series of molecular events that eventually lead to enhanced tumour expansion and aggressiveness caused by an overgrowth of the cancer cells with a reduced apoptosis and an increased angiogenesis. Suppressing the FABP5-related pathway via RNA interference against FABP5 has produced a 63-fold reduction in the average size of the tumours developed from CRPC cells in nude mice, a seven-fold reduction of tumour incidence, and a 100% reduction of metastasis rate. Experimental treatments of CRPC with novel FABP5 inhibitors have successfully inhibited the malignant progression of CRPC cells both *in vitro* and in nude mouse. These studies suggest that FABP5-related signal transduction pathway is a novel target for therapeutic intervention of CRPC cells.

Key words: FABP5; CRPC; fatty acids; PPAR γ ; tumorigenicity; metastasis

[§]These authors contributed equally to this work

Received: 18 June 2019; Revised: 19 July 2019; Accepted: 22 July 2019

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Fatty acid-binding protein 5 (FABP5) is a member of the fatty acid binding protein family that is responsible for transporting fatty acids between intracellular and extracellular membranes. FABP5 is a 15 kDa cytosolic protein, which binds with a high affinity to medium and long chain fatty acids.¹ Overexpression of FABP5 at the mRNA level was initially detected by specific molecular approaches developed for a systematic analysis of differential gene expression between benign and malignant human prostate epithelial cells.²⁻⁴ Using a 'subtractive selection' strategy combined with some other methods,⁵ a number of novel genes whose alternated expressions are involved in the malignant changes of the prostate cancer cells have been identified. Among them, FABP5 is the one studied most. After the FABP5 mRNA was identified as an upregulated gene in prostate cancer by differential expression analysis,⁶ the difference was further confirmed with Northern and slot blot analyses that showed that the expression levels of FABP5 mRNA in prostate cancer cell lines were increased by up to 17-fold from the benign epithelial cell line. At the protein level, the difference between the benign and the cancer cell lines was similar to that detected at the mRNA level. The level of FABP5 mRNA in prostate carcinoma tissues was significantly higher than that in the benign prostatic hyperplasia (BPH) by *in situ* hybridization. When the functional role of the increased FABP5 was characterized by gene transfection with the benign, non-metastatic rat Rama37 model,⁷ the forced expression of FABP5 in Rama37 cells produced metastasis in a significant (23% of the rats) number of animals, whereas no metastasis was produced in the control group. Both FABP5 mRNA and protein were overexpressed in the pool of FABP5-transfectants and in the sublines established from their metastases, no FABP5 was detected in the control transfectants. Immunocytochemical staining showed that FABP5 was expressed in both the primary tumours developed from the FABP5 transfectants and their metastases, it was not expressed in the primary tumours developed from the control that did not produce metastasis. Re-inoculation of the sublines established from metastases in Rama37 caused 50% of animals with metastases, which is higher than that obtained in the first-round inoculations. This result suggests that the metastatic clones have been preferentially selected from the original pool of a mixture of both metastatic and non-metastatic transfectant clones. When an archival set of prostate tissues was assessed,⁸ both nuclear and cytoplasmic FABP5 levels in carcinoma cells were significantly higher than those in normal and BPH tissues. The increased FABP5 was significantly associated with a reduced patient survival time. Thus, it was concluded that FABP5 was a metastasis-inducing gene and it promotes tumorigenicity and metastasis of cancer cells under suitable conditions.

FABP5-related signal transduction pathway in CRPC cells

Investigation of the molecular mechanisms involved in tumour-promoting activity of FABP5 has established that there is a fatty acid-initiated signalling pathway leading to malignant progression of prostatic cancer cells. Demethylation of the promoter region of FABP5 gene in CRPC cells causes a large-scale increase in FABP5 expression.⁹ Increased FABP5 expression plays a crucial role in this novel signalling pathway. When FABP5 expression is increased, large amounts of fatty acids are transported into the cancer cells and used as new sources of energy to meet the need of the quick cell growth. Excessive amounts of fatty acids are transported into the nucleus where they act as signalling molecules to stimulate their nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR γ). The activated PPAR γ then regulates the expression of its downstream genes to cause a chain of molecular events that eventually lead to an enhanced tumour expansion and an increased aggressiveness resulting from a reduced apoptosis and an increased angiogenesis.¹⁰⁻¹³

Recently, expression of FABP5, PPAR β/δ and PPAR γ has been measured in prostate cell lines, BPH and prostatic carcinomas to assess the correlation between FABP5 and either of the nuclear fatty acid receptors. The levels of both FABP5 and PPAR γ in prostate cancer cell lines are significantly higher than that in the benign cells. Their cytoplasmic and nuclear expressions are significantly increased in carcinomas than that in BPH tissues. The increased levels of FABP5 and PPAR γ are both significantly correlated to the increased joint Gleason scores (GS) of the cases and are significantly associated with a reduced patient survival time. The same study also showed that the enhanced expression of cytoplasmic FABP5 significantly correlated with an increased nuclear PPAR γ , but not related to the level of cytoplasmic PPAR γ . The expression of PPAR β/δ in carcinomas neither correlates with patient outcomes, nor associates with cytoplasmic or nuclear FABP5 level. Multivariate analysis suggested that FABP5 was independently associated with patient survival times, whereas PPAR γ was confounded by FABP5 in predicting patient survival times. Thus, both FABP5 and PPAR γ are suitable as prognostic factors to predict the survival of patients with prostate cancer. The increased FABP5 interacts with PPAR γ in a coordinated manner to promote malignant progression of the cancer cells.¹¹

To understand how the activated PPAR γ regulates its downstream possible cancer-promoting or tumour-suppressing genes, a study was conducted to investigate the molecular mechanisms involved in PPAR γ 's tumour-promoting role in prostate cancer. Suppression of PPAR γ in highly malignant PC3-M cells produced a significant reduction in the cell proliferation rate by up to 53%. It

inhibited the invasiveness by up to 89%, and reduced the number of colonies formed in soft agar by up to 94%. Knockdown of PPAR γ gene in PC3-M cells by siRNA significantly reduced tumour incidence in nude mice by 90%, the average tumour size by 99%, and prolonged the latent period significantly 3.5-fold. Results in this study combined with results from some previous studies suggested that FABP5 promoted VEGF expression and facilitated angiogenesis through PPAR γ that was activated by the simulation of the fatty acids transported by FABP5. Further investigations showed that upregulated VEGF expression by PPAR γ was achieved through binding to the PPAR-responsive elements in the promoter region of the VEGF gene in the cancer cells. Evidence also shows that androgen receptor (AR) can modulate VEGF expression through the Sp1/Sp3 binding site on the VEGF promoter in the cancer cells, this route, replaced gradually by the FABP5-PPAR γ -VEGF signal pathway, was disappeared as the cells gradually lost their androgen dependency. These results demonstrated that the FABP5-PPAR γ -VEGF signal transduction axis is a more important novel therapeutic target for angiogenesis-suppression treatment of CRPC than the androgen regulated route.¹⁴ It is possible that AR-initiated pathway plays an important role in androgen-dependent cancer cells, but the role of FABP5-related pathway increases gradually as the malignancy of the cancer cells increases, until it completely replaces the AR-initiated pathway when the cancer cells become completely androgen-independent. The growth and expansion of prostate cancer depend on androgen stimulation in the early stage. During androgen-deprivation therapy (ADT), the AR-initiated pathway is suppressed, cancer cells are subjected to high selection pressure, and most of them die. Under ADT conditions, some cancer cells may try to select for new sources of energy supply and some of these cells have survived ADT because they have successfully switched their energy source to fatty acids (fat degradation products). The FABP5-related pathway now replaces the AR-initiated pathway gradually with increasing malignancy or androgen-independency, and becomes the dominant pathway in AR-negative, androgen-independent CRPC cells. Thus, inhibiting the FABP5-related signal pathway is essential for the suppression of the malignant progression of CRPC cells.^{10,14} This model is very different from our previous understanding of the molecular mechanisms of how CRPC cells are transformed from androgen-dependent to independent status.¹⁵

Potential as a target for therapeutic intervention

A number of experimental treatments have been conducted on suppression of tumorigenicity and metastatic ability of the CRPC cells via inhibiting PPAR γ , and some encouraging results have been summarized by two recent reviews.^{16,17} To test the therapeutic potential

of targeting FABP5, a new cell line Si-clone-2 was established by using siRNA to interfere with FABP5 expression in highly malignant PC3-M cells. Suppression of FABP5 in highly malignant PC3-M cells significantly inhibited their proliferation and tumorigenicity *in vitro*. When the FABP5-suppressed cell line Si-clone-2 was tested in nude mice, only two out of 13 mice developed very small primary tumours, with an average size of 23 mg, without any metastases. But all 14 mice in the control group developed primary tumours with an average size of 1450 mg, and 64.3% (9/14) animals produced metastasis. When inoculated subcutaneously, all five mice in the control group developed tumours from day 4, with an average size of 1471 mm³ at 5 weeks after the inoculation; whereas Si-clone-2 cells produced no tumours at any time-point in any of the five animals, indicating the suppression occurred at the initiation stage. Thus the internally transcribed siRNA within cells produced a 63-fold reduction in average tumour size, seven-fold reduction in tumour incidence, and 100% reduction in metastasis rate.⁸ To test whether siRNA to FABP5 delivered to the external environment of a CRPC would suppress tumorigenicity *in vivo*, experiments were established whereby siRNA to FABP5 suspended in a RNA-stabilizing substance atelocollagen was injected around tumour masses produced by PC3-M cells in nude mice and compared to the effect of injections of non-specific scrambled siRNA in atelocollagen. At autopsy, the average size of tumours from the groups treated with 10 and 15 μ M siRNA in atelocollagen was significantly smaller by more than three-fold that of the control. These data demonstrate that FABP5 siRNA delivered by atelocollagen to the external environment surrounding a tumour mass can effectively inhibit the CRPC cell growth in nude mice. However, using siRNAs directly as treatment reagents is much less effective than the internally transcribed siRNA, it can only slow down, rather than stop the tumour growth. Furthermore, stabilizing agent atelocollagen (a cow skin extract) is too expensive to be available in routine supply.¹⁸

A recent experimental CRPC treatment by suppressing the FABP5-related pathway has been conducted by direct application of a chemical inhibitor SB-FI-26 to suppress the biological activity of FABP5.¹⁹ SB-FI-26 can significantly suppress the invasiveness, migration, proliferation, and the anchorage-independent growth of PC3-M cells *in vitro*. It suppressed the metastatic rate by 50% and the average primary tumour mass developed from the prostate gland of the nude mice by nine-fold. The inhibitor SB-FI-26 competitively binds to FABP5 to inhibit the fatty acid uptake by the cancer cells, and hence avoids fatty acid stimulation of PPAR γ . This prevents PPAR γ activating the downstream regulated cancer-promoting genes. SB-FI-26 is, in fact, the main component of the Chinese traditional herbal medicine *incarvillea sinensis*, which has been used as an analgesic to treat rheumatism for hundreds of years.²⁰ It has been suggested to be a candidate for development of

anti-inflammatory and anti-nociceptive drugs.²¹ The main problem in using SB-FI-26 as an experimental therapeutic agent for CRPC is the issue of dosage. For achieving the optimal effect, the dose (>100 µM) required is too high to be practical to maintain a sufficient supply. How to increase its effect and hence to reduce the dose of SB-FI-26 needed for CRPC treatment is a new challenge.

More recently, a highly efficient recombinant FABP5 inhibitor, named dmrFABP5, has been produced to conduct experimental treatment of CRPC.²² Treatment with dmrFABP5 significantly suppressed the proliferation, migration, invasion and soft agar colony formation of the highly malignant CRPC cells PC3-M *in vitro*. To test dmrFABP5's suppressive effect on CRPC, the human PC3-M cells were implanted orthotopically into the prostate gland of immunosuppressed nude mice to produce tumours. Treatment with dmrFABP5 has produced a highly significant inhibition of 100% in metastatic rate and a highly significant reduction of 13-fold in the average size of primary tumours. Immunocytochemical analysis showed that the staining intensity of dmrFABP5 treated tumours was reduced by 67%. Similar to SB-FI-26, dmrFABP5 suppressed the cancer cells by blocking fatty acid stimulation of PPAR γ , and thereby prevented it activating downstream genes. The difference between dmrFABP5 and SB-FI-26 is that dmrFABP5 did not bind to FABP5 to suppress the cellular fatty acid uptakes. Thus, more study is required to understand exactly how dmrFABP5 suppressed PPAR γ . The novel FABP5 inhibitor dmrFABP5 is the most effective molecule discovered so far for experimental treatment of CRPC and its inhibitory effect is much greater than that produced by SB-FI-26.

Discussion

We have shown that the FABP5-related signal pathway is a newly identified signal transduction route in CRPC cells and the evidence in experimental treatment showed that suppressing this pathway can effectively inhibit the malignant progression of the CRPC cells. Although no clinical trial has been conducted yet in human patients to study the treatment effect of the inhibitors of the FABP5-related pathway, it is reasonable to believe that at least some of this pathway inhibitors are well tolerated in human body and can act effectively as drugs. This optimistic view is based on the fact that the FABP5 inhibitor truxillic acid is the main component of the herbal medicine *incarvillea sinensis*, which was used as an analgesic to treat rheumatism in traditional medicine,²⁰ and it is also a candidate for development of anti-inflammatory and anti-nociceptive drugs.²¹ Thus, this pathway is a possible novel target for therapeutic intervention. It must be emphasized, however, that CRPC is a complicated disease and it may contain many different, yet currently unknown subtypes, including a mixture of FABP5-positive, FABP5-negative, AR-positive, and AR-negative cases. Thus, a personalized medicine approach will have to be undertaken for each patient

to identify into which of these four possible classes individual tumours will fall. These classes can be relatively easily identified from immunohistochemical analysis of standard pathological specimens of the primary tumours. However, in the initial stages it will be necessary to relate their presence to their functional capability. Thus, primary cultures of each of these four prostate cancer subtypes will have to be obtained and tested with inhibitors of the FABP-dependent and AR-dependent pathways singly and in combination to ascertain how the individual patient's prostate cancer cells will respond to treatment. It may be that the single inhibitors will only be required for the pure FABP5-positive and AR-positive groups, whereas a combination of both inhibitors may be required for the mixed group and neither inhibitor either singly or in combination may be useful in the purely negative group. Thus, more work is needed to clarify whether the FABP5-related pathway exists in only a proportion of CRPC cases, or in all CRPC cases, before true clinical trials of inhibitors to this pathway can be undertaken.

Since FABP5 has been identified as a cancer-promoting molecule in prostate cancer,^{6,12} subsequent studies have shown that increased FABP5 expression is now associated with most²³⁻²⁷ commonly occurring human cancers. It will be interesting to know whether suppressing the FABP5-related pathway can also inhibit the progression of other FABP5-positive cancers so generalizing the concept and approach beyond that of prostate cancer.

Acknowledgements

A.A.N. and S.A.A. are Saudi Arabia Government PhD Scholarship holders. The research work of Y.K.'s team has been supported by grants from North West Cancer Research, Wellcome Trust, US Army Fund, MRC, Cancer and Polio Research Fund, and Prostate Cancer UK.

Conflict of interest statement

None declared.

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