Expression of cutaneous fatty acid-binding protein (C-FABP) in prostate cancer: Potential prognostic marker and target for tumourigenicity-suppression

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Received November 20, 2007; Accepted December 31, 2007

Abstract. C-FABP or E-FABP is a metastasis inducing gene over expressed in human prostate carcinomas. To study its prognostic significance, an archival set of prostate tissues was analysed immunohistochemically. Levels of both nuclear and cytoplasmic C-FABP expression in carcinoma cells were significantly higher than those in normal and BPH tissues and the increased C-FABP was significantly associated with a reduced patient survival time. To test the therapeutic potential of targeting C-FABP, a clone (Si-clone-2) of cells was established by interfering C-FABP expression in highly malignant PC-3M cells. Suppression of C-FABP in cancer cells significantly inhibited their proliferation and tumourigenicity in vitro. When Si-clone-2 cells were orthotopically implanted into the prostate gland of mouse, 2/13 mice produced primary tumours with an average size of 23±5 mg, and no metastasis was produced in any of the 13 animals. Whereas in the control group, all 14 mice produced primary tumours with an average size of 1450±370 mg and 9/14 (64.3%) produced metastasis. When inoculated subcutaneously, all 5 mice inoculated with control cells developed tumours from day 4, with an average size of 1471±544 mm³ at 5 weeks after the inoculation; whereas Si-clone-2 cells produced no tumours in any of the 5 animals at any time-point, indicating the suppression occurred at the initiation stage. Our results suggest that C-FABP may be used as a potential prognostic marker to predict patient outcome and the increased C-FABP expression is a possible target to inhibit the malignant progression of prostate cancer cells.

Introduction

Prostate cancer is an important male disease in the developed world and more than 30,000 men died each year from this cancer in the USA alone (1,2). Like other cancerous diseases, the causes for the development and metastasis of prostate cancer may involve many complicated molecular events, including the increased activities of cancer-promoting genes and the diminished activities of cancer-suppressing genes (3,4). Thus, suppression or promotion of expression of the cancer-related genes may provide an effective way to interfere with the malignant progression of cancer cells. Using several differential display (5)-derived molecular approaches (6-8) combined with a 'subtractive selection' strategy, we have identified and characterised several genes (9,10) whose altered expression may contribute to the initiation and development of prostate cancer. One of such genes involved in malignant progression of cancer cells (11) is that coding for human cutaneous fatty acid-binding protein (C-FABP) which is also called E(EPIDEMIC)-FABP or PA(Psoriasis-associated)-FABP, and was originally identified in human skin (12).

In our previous work, it was demonstrated that C-FABP was over-expressed in human prostate cancer cell lines and in most of the prostate carcinoma tissues. When C-FABP gene was transfected into the benign Rama 37 model cells (13), it could promote the growth of primary tumours and induce malignant dissemination (14,15). It was also demonstrated that C-FABP induced metastasis through up-regulation of other oncogenes such as vascular endothelial growth factor (VEGF) (15,16). Although the role of C-FABP in malignant progression of prostate cancer cells had been characterised and confirmed, the possibility to use the increased expression of C-FABP as a prognostic factor was not explored. The therapeutic potential of targeting the over-expressed C-FABP in the highly malignant prostate cancer cells was not previously investigated.

In this work, we have carefully analysed the expression status of C-FABP in an archival set of human prostate tissues.
with patient-following up data and assessed the relationship between the increased C-FABP expression in carcinoma tissues and the length of patient survival time. We have also related the elevated expression levels of C-FABP with the increased joint Gleason scores (GS). To test the therapeutic potential, we have suppressed the expression of C-FABP in the highly malignant prostate cancer cell line PC-3M (17) by RNA interference (RNAi) (18,19) to investigate whether suppression of C-FABP expression can inhibit the onset and the development of primary tumours and their ability to metastasize to secondary sites in mouse.

Materials and methods

Tissue samples and patients' data. Human prostate tissues comprised an archival set with follow-up data held within the diagnostic archive in the Department of Pathology, University of Liverpool, UK. The patients who died from causes other than metastatic prostate cancer have been excluded from this study. Tissues were taken from 70 prostate adenocarcinoma patients with an average age of 73 years and from 35 benign prostatic hyperplasia (BPH) patients with an average age of 67.5 years who were treated by trans-urethral resection of prostate (TURP) in the Royal Liverpool University Hospital during the 5-year period of 1995-2000. The 7 normal prostate tissues were taken from road accident victims with an average age of 48 years who did not have a history of prostatic disease. This study was approved by Liverpool Local Science Ethics Committee in accordance with the Medical Research Council guidelines (project reference no.: Ke: 02/019). Tissue sections were examined independently by two observers and classified as normal, BPH, and carcinomas. The carcinomas were further classified according to their combined GS (20).

Histological and immunohistochemical staining of tissues. Formalin-fixed tissues were processed and embedded in paraffin wax. Histological sections (4-μm) were cut and mounted onto slides as described previously (21). The tissue sections were incubated at 37°C overnight prior to staining with haematoxylin and eosin using an automated Varistain sections were incubated at 37˚C overnight prior to staining as described previously (21). The tissue

Designing RNA interference molecule to silence C-FABP. A unique 19nt sequence (ACTCAGACTGTCTGCAACT) located towards the end of the 5-prime of C-FABP gene was chosen as the siRNA target site, using the method described on the biotech company Ambion (Cambridgeshire, UK) website (www.ambion.com). This site was immediately downstream from an AA dinucleotide sequence. The uniqueness of the 19nt sequence was confirmed by running the chosen sequence in a blast search to ensure there was no significant sequence homology towards other human or mouse sequences. The silencing construct consisted of 2 complementary oligonucleotides orientated in sense and antisense directions. The 2 DNA strands were the same 19 nucleotide sequence in sense (5'-ACTCAGACTGTCTGCAACTTTCAAGAGAAATGAGACAGCTGAGTGTGGTTTTT-3') and antisense orientations separated by a 9 nucleotide loop sequence (TTCAGAGA) in the middle and capped at the 3'-end with a poly T termination sequence. This short DNA molecule was flanked at the 5'-end by a poly A termination sequence preceded by an EcoRI restriction enzyme sequence. The 3'-end of the antisense strand was capped by an ApaI restriction sequence. The short DNA was commercially synthesised (Invitrogen Life Technologies, Strathclyde, UK) and purchased as two separate oligonucleotides. The sense and antisense strands were annealed together to form a single double stranded DNA molecule which was then ligated to a pSilencer 1.0 U6 plasmid (Ambion, Cambridgeshire, UK) the EcoRI and ApaI sites, and amplified by molecular cloning and transforming into E. coli (DH5-α) cells. The orientation of the insert was confirmed by automated DNA sequencing using an internal pSilencer 1.0 U6 T3 specific primer (5'-ATTACC CTCAAGTAAGGGA-3').

Transfection of siRNA C-FABP silencing construct. The SiRNA-C-FABP silencing construct was transfected into PC-3M cells by calcium phosphate co-precipitation technique as described previously (24,25). Exponentially growing PC-3M cells were seeded in 2 Petri dishes (9 cm in diameter) at a density of 0.5x10^6/dish. One dish was co-transfected with 10 μg pSV2neo plasmid DNA plus 10 μg pSilencer plasmid DNA to form a pool of control transfectants; the other dish was co-transfected with 18 μg siRNA silencing construct DNA plus 2 μg pSV2-neo DNA to generate C-FABP suppressed
clones. Cells were passaged at a 1:10 ratio, and transfected cells were selected by supplementation of RPMI-1640 medium with 0.5 mg/ml geneticin G418 (Invitrogen, Paisley, UK); the medium was replaced every 3–4 days. When cell colonies had grown to approximately 3 mm in size, they were isolated by ring-cloning. Five colonies were isolated for selections of lowest C-FABP expressor. In a separate transfection, a pool of control transfectants was generated.

Detection of protein expression in cultured cells. The expression of C-FABP at protein level in cultured cells was detected by Western blot analysis using an ECL detection system as described previously (26). Total cellular protein was extracted from different cell lines with 2X SDS lysis buffer. Proteins were separated by SDS-PAGE in 10% acrylamide gel, and transferred onto a nitrocellulose membrane (Hybond ECL, Amersham Pharmacia, Amersham, UK). Primary antibody immunoblotting was performed on proteins using rabbit anti-human C-FABP (Iwaki Glass Corp.) at 1:200 dilution, followed by horseradish peroxidase-conjugated swine anti-rabbit IgG (Dako) diluted at 1:1000. The protein bands were visualized by exposure to Kodak XAR-5 film and quantified by measuring the intensity of the peak areas using Alpha Imager 2000 densitometer (Alpha Innotech, Cannock, UK). Constitutively expressed actin protein was detected to correct possible loading errors.

Proliferation assay of siRNA transfectants. Effect of suppressed C-FABP expression on cellular proliferation was measured by a proliferation assay. The lowest C-FABP expressor (Si-clone-2), two moderate C-FABP expressors (Si-clone-3 and Si-clone-4), and the control transfectants were cultured for 24 h in RPMI-1640 medium supplemented with 4% (v/v) fetal calf serum, penicillin (100 U/ml) and streptomycin (100 mg/ml) under 5% CO2 at 37°C. Exponentially growing cells from each culture were seeded in 7 triplicates at a density of 2x105 cells/ml. The mixture was then poured onto the top of the preset basement gel layer and incubated at 4°C for solidification. Once set, the cells in the plates were incubated at 37°C under 5% CO2 for 6 weeks. The colonies developed in each well were counted and the tumourigenicity of each cell line was decided by the number of colonies formed in LMA-RPMI mixture within the 6-week experimental period.

Nude mouse assay. To test for tumourigenicity and metastasis, Si-clone-2 and the control transfectants were orthotopically implanted (28) into the prostate gland of mouse. Fourteen mice were implanted with Si-clone-2 and control cells, respectively, into the dorso-lateral lobe of prostate gland of the immuno-incompetent CD-1 nude mice (Harlan, UK) at a density of 2x106 cells/20 μl PBS. To implant the cells, each mouse was anaesthetised with 0.2 mg/kg/sc Buprenorphine (tremgesic) analgesia, and anaesthetised using gas induction at 50%/50% oxygen/nitrous oxide with 1-2% isoflurane and oxygen, and nitrous oxide anaesthetic gas. Mice were placed in the supine position, swabbed with iodine around the abdomen, hindquarters and genital area. A midline incision was made in the lower abdomen. Using the bladder as orientation, the dorso-lateral lobe of the mouse prostate gland was identified for implantation of control and Si-clone-2 cells. Following injection of cells, the surgical wound was sutured using single stitch sutures and the animals monitored post-operatively for loss of appetite, poor grooming or general malaise. Mice were sacrificed 5 weeks after inoculation. At autopsy, primary tumours (where available) were dissected along with the lungs, liver, heart, lymph nodes and other organs of each mouse. Each primary tumour was weighed before fixation in 10% formalin for a period of 24 h. To find out at what point the suppression activity starts, Si-clone-2 and control transfectant cells were inoculated subcutaneously into the shoulder region of nude mice at a density of 2x106 in 0.2 ml of PBS. The growth of tumour at the site of inoculation was measured weekly over a period of 5 weeks. Measurements of tumour volume was calculated by the formula: \( \text{Volume} = \frac{\text{length} \times \text{width} \times \text{height}}{2} \) (29). All animal experiments were conducted under UKCCCR guidelines with Home Office Project Licence PPL 42270 to Professor Y. Ke.

Statistical analysis. Correlation between C-FABP staining and the nature of prostate tissues (normal, benign or malignant) was assessed by 2-sided Fisher's exact test and \( \chi^2 \) analysis, using the Statistical Package for Social Sciences, Version 14.0 (SPSS Inc., Chicago, IL, USA). Association between C-FABP staining and GS of carcinomas was assessed by \( \chi^2 \) test. Correlation between the increased C-FABP staining and the length of prostate cancer patient survival were evaluated using Kaplan-Meier analysis. Differences between patients' groups were assessed by log-rank test. Statistical significance was defined as \( \rho<0.05 \).

Results

C-FABP expression in prostate tissues. Results of immunohistochemical staining for C-FABP in prostate tissues are shown in Table I. Neither cytoplasmic nor nuclear staining...
was observed in any of the 7 normal prostate tissues. The expression of C-FABP in BPH and carcinoma tissues was detected in both cytoplasm and nucleus. Amongst 35 BPH cases, 33 (94.3%) were unstained and 2 (5.7%) had weak cytoplasmic staining. Weak nuclear staining was observed in 7 (20%) BPH cases, moderate and strong nuclear staining was observed in 5 (14.2%) and 3 (8.57%) respectively, and 20 (57.1%) were unstained. Among 70 adenocarcinomas, 67 (96%) showed positively stained cytoplasm, of which 18 (26%) stained weakly, 39 (56%) stained moderately and 10 (14%) stained strongly. Nuclear staining was negative in 11 (16%), weakly positive in 14 (20%), moderately positive in 22 (31%) and strongly positive in 23 (33%) carcinomas. The intensities of both cytoplasmic ($\chi^2$ test, p<0.001) and nuclear ($\chi^2$ test, p<0.001) C-FABP staining in carcinomas were significantly higher than those observed in normal and BPH cases. Some representative stains are shown in Fig. 1.

**Correlation of C-FABP expression to GS.** To correlate the intensity of C-FABP expression and GS, carcinomas were divided into low (≤5), moderate (6-7) and high (8-10) GS groups. The C-FABP expression status in the 3 groups of carcinomas is shown in Table I. The increased cytoplasmic staining intensities of C-FABP were significantly correlated with the increased GS of the carcinomas (2-sided Fisher's exact test, p<0.001) but the correlation between the increased nuclear staining intensities and the increased GS was not significant (2-sided Fisher's exact test, p>0.5).

**Relationship between patient survival and C-FABP expression.** The relationship between C-FABP expression and length of patient survival time was assessed by Kaplan-Meier survival analysis. The relationship between the cumulative probability of surviving (survival time following initial diagnosis) and C-FABP expression is shown in Fig. 2A (cytoplasmic) and B (nuclear). The median survival time for the patients with negative and weakly cytoplasmic C-FABP staining (group 1) was 80 months; whereas the median survival time for the patients with moderate and strong cytoplasmic C-FABP staining (group 2) was significantly reduced to 36 months (log-rank test, p<0.003). The median survival time for cases

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cytoplasm Stain intensities</th>
<th>Nuclear Stain intensities</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BPH (total)</td>
<td>33</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Scores≤5</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Scores6-7</td>
<td>0</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Scores8-10</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

*Combined Gleason scores.

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Table I. CFABP cytoplasmic and nuclear expression in normal, benign and malignant human prostate.
with no nuclear staining (group 1) was 80 months, compared to a significantly (log-rank test, p=0.01) reduced 48 months in cases with positive nuclear staining (group 2).

C-FABP expression in control and different Si-transfectant clones. The results of Western blot analysis of C-FABP expression in the control transfectants, which were generated by transfecting the parental PC-3M cells with plasmid DNA alone, and in 4 different Si-transfectant clones are shown in Fig. 3A. Further quantitative analysis of the relative levels of C-FABP expression in the control cells and in different Si-transfectant clones is shown in Fig. 3B. When the level of C-FABP expressed in the control transfectants was set at 1, the levels of C-FABP expression in all transfectant clones (Si-clone-1-4) were reduced to 0.71 (Si-clone-1), 0.032 (Si-clone-2), 0.56 (Si-clone-3) and 0.69 (Si-clone-4) respectively. Si-clone-2 was identified as the least C-FABP expressor, in which the siRNA produced a 31-fold reduction in level of C-FABP when compared to the control.

The effect of siRNA on cell proliferation. The proliferation rates of the control and Si-clone-2 to 4 transfectant cells measured by proliferation assay are shown in Fig. 4A. After the same numbers (1x10^3) of cells from each cell line were cultured in separate plates for 6 days, the number of cells from each of the 3 Si-clones (2-4) was 35,000±5,000, 59,000±6,000, and 68,000±5,000, respectively. In contrast the number of cells derived from the control transfectants was 102,900±13,000. In comparison with the control cells, the number of Si-clone-2 cells was significantly (Student's
as the total number of animals with tumours/the total number of animals inoculated. cTumour mass was the weight at autopsy. The results shown are the mean (± SE) of each group. dIncidence of metastases was defined as the total number of mice with metastasis/the total number of animals inoculated.

### Table II. Incidence of primary tumours and metastases produced by the control and the Si-clone-2 implanted orthotopically into the mouse prostate gland of nude mice.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Surgical procedures</th>
<th>Tumour incidence (%)</th>
<th>Mean tumour mass (mg)</th>
<th>Metastasis incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Orthotopic implantation</td>
<td>13/14 (93)</td>
<td>1450±370</td>
<td>9/14 (64%)</td>
</tr>
<tr>
<td>Si-Clone-2</td>
<td>Orthotopic implantation</td>
<td>2/13 (15)</td>
<td>23±5</td>
<td>0/13 (0%)</td>
</tr>
</tbody>
</table>

*One mouse in the Si-clone-2 group suffered post-operative complications and was excluded from the study. Tumour incidence was defined as the total number of animals with tumours/the total number of animals inoculated. Metastasis was the weight at autopsy. The results shown are the mean (± SE) of each group. Incidence of metastases was defined as the total number of mice with metastasis/the total number of animals inoculated.**

Tumourigenicity and metastasis assay in nude mouse. The tumourigenicity and metastatic capability of the control and the Si-clone-2 cells implanted orthotopically into the mouse prostate gland are shown in Table II. Each of the 2 cell lines was inoculated in 14 animals. But 1 mouse in the test group (inoculated with Si-clone-2 cells) suffered post-operative complications and was excluded from this experiment. Thirteen out of 14 (93%) mice implanted with control transfectant cells produced primary tumours, whereas only 2 of the 13 (15%) mice implanted with Si-clone-2 cells produced primary tumours, a significant (Student's t-test, p<0.02) reduction from control. The average size of tumours produced by control cells was 1450±37 mg. This was significantly (Student's t-test, p<0.0001) reduced to only 23±5 mg produced by Si-clone-2 cells. Nine of the 14 mice implanted with control transfectants developed metastases and all were found in the lungs. Seven metastases were micro-lesions and 2 were macroscopically visible. Histological examination revealed a haematogenous route of spread of the malignant cells (Fig. 5A). No metastasis was produced in any of the mice implanted with Si-clone-2 cells. Immunohistological staining showed that C-FABP was strongly expressed in both primary tumours and metastases produced by the control cells. It was also strongly expressed in primary tumours produced by Si-clone-2 cells (Fig. 5B).

To test whether the suppression of tumour development in Si-clone-2 cells occurred in the initiation stage or in the maintenance stage, the control and Si-clone-2 cells were subcutaneously inoculated into 2 groups (5 each) of mice. The average volume of tumours produced by 2 groups of animals over a 5-week period is shown in Fig. 6. All 5 mice (100%) inoculated with control transfectants developed visible tumours after a 4-day latent period. When the animals were sacrificed 5 weeks after inoculation, the mean volume of the 5 tumours...
was 1471±997 mm³. In the group of mice inoculated with Si-clone-2 cells, none of the animals (0%) developed tumours. The tumour incidence in this group of mice was significantly (Student’s t-test, p<0.025) lower than that in the control group.

Discussion

C-FABP is a 15-kDa cytosolic protein of the fatty acid binding protein family (12), which binds with high affinity to long chain fatty acids. C-FABP expression, in addition to skin, has been identified in the endothelial cells of the placenta, heart, skeletal muscle, small intestine, renal medulla and in the Clara and goblet cells of the lung (30), and also in malignancies of the bladder and pancreas (31-33). Although the molecular mechanism by which increased C-FABP activity functions within these tissues is unclear, its ability to bind and transport fatty acids may be of importance in the malignant transformation of some cancer cells. Our previous work, has found that the C-FABP gene was overexpressed in malignant breast and prostate epithelial cell lines. When C-FABP gene was transfected into the benign rat Rama 37 model cells (24,25) and the transfectants subsequently inoculated into Wistar Furth rats (Wistar OLA strain), a significant number of animals developed metastasis (11). Further studies demonstrated that C-FABP promoted tumourigenesis and metastasis of prostate cancer cells by facilitating angiogenesis through up-regulating VEGF gene (34,35). Thus it was suggested that there may be a fatty acid signalling-initiated pathway that leads to metastasis in prostate cancer, and C-FABP, VEGF, and the peroxisome proliferator-activated receptor (PPAR)-γ (36) are all important factors in this possible pathway (14,15).

Although over-expression of C-FABP and its involvement in malignant progression of prostate cancer and other cancers has been reported (14,32,33,37,38), its possible prognostic significance and usefulness as a therapeutic target were not investigated. In order to study the clinical relevance of the elevated C-FABP expression in prostate cancer, we examined the expression status of C-FABP in an archival set of prostate cancer tissues. It was found that only 5.7% of the BPH samples expressed C-FABP, whereas in carcinomas, 96% of the tissues expressed C-FABP (Table I). Although there was no significant correlation between the increased nuclear C-FABP staining and the increased GS, the increased cytoplasmic intensities of C-FABP staining were significantly correlated with the increased GS of the carcinomas (2-sided Fisher’s exact test, p<0.001), indicating that the increased C-FABP expression in cytoplasm might contribute to an increased...
degree of malignancy. The median survival time for the patients with moderate and strong cytoplasmic C-FABP staining was 36 months, whereas for the patients with negative and weakly cytoplasmic C-FABP staining the median survival time was 80 months (Fig. 2A). Thus, the increased C-FABP in cytoplasm was significantly associated with a 2.2-fold reduction in patient survival (log-rank test, p<0.003). The median survival time for cases with no nuclear C-FABP staining was 80 months, whereas that for cases with positive nuclear stains was 48 months, a significant reduction of 1.7 times (log-rank test, p=0.01). The median survival time was 120 months for the cases in which C-FABP expression in cytoplasm and nucleus jointly was weak or absent; reduced to 80 months for the cases expressing moderate levels of C-FABP in either cytoplasm or nucleus; and further highly significantly (log-rank test, p<0.0001) reduced to 24 months for cases strongly expressing C-FABP. These results suggested that the increased C-FABP in cytoplasm and nucleus, either separately or jointly, was significantly associated with a reduced patient survival time. Therefore, an increased C-FABP expression in prostate cancer may be a new prognostic factor for prediction of patient outcome.

To explore the possibility of using C-FABP expression as a target to inhibit the malignant progression of prostate cancer cells, we have successfully suppressed the C-FABP expression in the highly malignant, metastatic prostate cancer cell line PC-3M and generated a transfectant clone (Si-clone-2), in which the level of C-FABP was suppressed by 31-fold. In addition several more clones producing moderately-reduced levels of C-FABP were also established. When tested by a proliferation assay for 6 days, Si-clone-2 cells exhibited a significantly lower (by 2.5-fold) proliferation rate (Fig. 4A). Si-clone-3 and Si-clone-4, which expressed 0.56- and 0.69-times of the amount of C-FABP than in control, exhibited lower (by 1.7- and 1.5-fold, respectively) proliferation rates. These results suggested a trend for reduced proliferation to be associated with greater inhibition of C-FABP expression. A similar trend was also observed in soft agar assays. Thus, while the control cells expressed 31-, 1.8- and 1.5-fold more C-FABP respectively than Si-clone 2 to 4 cells, they produced 2.3-, 1.5- and 1.2-times the number of colonies (Fig. 4B). Thus, suppression of C-FABP expression in highly malignant prostate cancer cells in vitro greatly inhibits their proliferation rate and tumourigenicity.

When the control and the Si-clone-2 cells were implanted orthotopically into the mouse prostate gland, 13/14 (93%) mice in the control group produced primary tumours, whereas only 2 of the 13 (15%) mice implanted with Si-clone-2 cells produced primary tumours, a significant (Fisher's exact test, p<0.02) 6.2-fold reduction in tumour incidence. The average size of the tumours produced by the control cells was 1450±37 mg, compared with 23±5 mg produced by Si-clone-2 cells, a significant (Student's t-test, p<0.0001) 63-fold reduction (Table II). Thus, suppressing the expression of C-FABP in PC-3M cells significantly reduced both the incidence and average size of tumours growing in the primary inoculation site. Further analysis showed that 9/14 (64%) mice implanted with control transfectants produced metastases, whereas no metastasis occurred in any of the mice implanted with Si-clone-2 cells.

The role of C-FABP as a promoter of the development of both primary tumours and metastasis was confirmed by our observation of strong immunoeexpression in both primary tumours and metastases derived from control cells (Fig. 5B). All 5 mice (100%) inoculated subcutaneously with control cells developed visible tumours after a 4-day latent period. When the animals were sacrificed 5 weeks after inoculation, the mean volume of the 5 tumours was 1471±997 mm³, whereas no tumours developed in any of the 5 animals inoculated subcutaneously with the control cells. This result suggested that the suppression of tumour development in Si-clone-2 cells occurred in the initiation stage by preventing the cells from forming primary tumours. Interestingly, C-FABP was expressed in the 2 small primary tumours produced by Si-clone-2 (Fig. 5B). This result combined with subcutaneous inoculation experiment results indicated that the development of these 2 tumours might be consequences of de-suppression of C-FABP in Si-clone-2 cells. During the in vivo inoculation, it might be possible that some changes occurred to the mechanism, by which the RNAi-suppressed the expression of C-FABP, and as a result of these yet unknown changes, the suppression mechanism was loosened and the expression of C-FABP was recovered in the cancer cells, and hence the tumours developed.

An increased activity of the cancer-promoting genes is one of the major factors involved in the development and spread of the cancer cells. The increased expression of these cancer-promoting genes provides a valuable pool of candidates, from which reliable prognostic markers may be selected (38). Furthermore, suppression of the expression of cancer-promoting genes may provide an effective way to stop or to reverse malignant progression (40-42). However, the success of the strategies for targeting metastasis-promoting genes will first depend on the identification, verification and determination of reliable targets. In this work, we have assessed whether C-FABP maybe such a target. Our results show that an increased C-FABP expression is significantly associated with the early demise of prostate cancer patients, whereas suppressing the expression of C-FABP in prostate cancer cells results in a highly significant reduction in tumourigenicity and complete inhibition of metastasis. These finding suggest that increased expression of C-FABP maybe a valuable prognostic factor predicting the outcome in prostate cancer patients, and it may also prove to be an important target for designing effective strategies to treat the disease.

Acknowledgements

We thank North West Cancer Research Fund for its generous support of research grant.

References


