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Expression and Sub-Cellular Localization of the CCAAT/Enhancer Binding Protein α in Relation to Postnatal Development and Malignancy of the Prostate

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BACKGROUND. C/EBPα is a critical mediator of terminal differentiation and a tumor suppressor through its strong antiproliferative actions on cell cycle regulatory proteins. C/EBPα also appears to regulate androgen receptor (AR) AR signaling. There, is a paucity of information on the expression and sub-cellular localization of C/EBPα in normal mouse and human prostate and in prostate cancer.
METHODS. Immunohistochemistry of tissues including tissue arrays, quantitative polymerase chain reaction and mRNA expression database mining.
RESULTS. In the mouse prostate epithelium, C/EBPα was present at 1 week postnatal localized in the cytosol, began to show nuclear localization at 8 weeks and continued to show prominent nuclear expression at 10 weeks and beyond; C/EBPα mRNA was expressed at all ages. In humans, C/EBPα showed prominent nuclear localization from peripubescence up to middle age but was sequestered in the cytosol in older individuals; the mRNA level for C/EBPα remained essentially unchanged. Most prostate adenocarcinomas expressed a range of levels of C/EBPα mRNA and protein that were relatively high in metastatic tumors in a manner that correlated with AR expression; however, most cells showed C/EBPα sequestered in the cytosol.
CONCLUSIONS. Temporal changes in sub-cellular localization of C/EBPα are consistent with a role in prostate differentiation and as a prostate tumor suppressor; the cytoplasmic sequestration of C/EBPα, unique to older human prostates, is arguably a permissive condition for the greater frequency of proliferative disorders of the prostate. In malignant prostate C/EBPα may be available to regulate AR signaling through transient changes in its sub-cellular localization. *Prostate 68: 1206–1214, 2008.*

KEY WORDS: **prostate; C/EBPα; androgen receptor**

INTRODUCTION

The CCAAT enhancer binding protein (C/EBP) family comprises at least six members, named α, β, γ, δ, ε, and ζ [1]. They are homo- or hetero-dimeric basic/leucine zipper transcription factors that recognize the CCAAT enhancer, a divergent dyad repeat sequence RTTGCGYAAY, in which R and Y represent A/G and C/T respectively [2]. Members of the C/EBP family are required for the differentiation of adipocytes, myeloid cells, hepatocytes and other cell types [1]. Among C/EBP proteins, C/EBPα is distinctive in that in addition to its transcriptional activity, it inhibits cell proliferation by several non-genomic mechanisms [3–5]. C/EBPα can exert its antiproliferative actions without binding to DNA [6] through protein–protein

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interactions; they include stabilization of p21 [7,8], disruption of E2F complexes [9–11], inhibition/degradation of cdk2 and cdk4 [12,13] and interaction with the SWI/SNF chromatin remodeling complex [14].

The antiproliferative actions of C/EBPα cause it to be a tumor suppressor in several cell types such as acute myeloid leukemia, lung cancer, hepatoma, breast cancer, and skin cancer [5,15–21]. However, in liver tumors, dephosphorylation of C/EBPα by activation of the PI3K/AKT pathway inhibits its interactions with cdk2 and E2F complexes [22]; dephosphorylated C/EBPα may contribute to proliferation by sequestering Rb [23]. Since C/EBPα is also frequently expressed in malignant tissues (Ref. [24] and Oncomine microarray data repository; http://www.oncomine.org/), an altered phosphorylation state could be expected to cause the protein to support tumor proliferation [23].

There is some evidence that in both humans and rodents, C/EBPα is expressed in prostate epithelial cells [25,26] and DNA microarray data indicates the presence of mRNA for C/EBPα in malignant human prostate tissue [27]. C/EBPα has also been reported to associate with the androgen receptor (AR) [26] suggesting a role in regulating AR signaling. Ectopic C/EBPα was antiproliferative in C/EBPα-negative prostate cancer cells. Since C/EBPα could thus play a role in normal prostate development and also in the physiology of prostate tumors, there is currently a need for a systematic investigation of its regulation during various stages of the development of the normal human and mouse prostates and in a spectrum of prostate tumors. This study reveals unique and physiologically significant aspects of C/EBPα expression in prostate tissues.

MATERIALS AND METHODS

Immunohistochemistry of Mouse and Human ProstateTissues

Black/6 mice were euthanized at specific ages ranging from 1 week to 8 months. The prostates were dissected immediately after euthanasia and fixed in formalin and embedded in paraffin. Sections were stained for C/EBPα using standard procedures. Briefly, antibody to C/EBPα (sc-61, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was titrated on normal mouse prostate. Rat and mouse liver were used as positive controls. Unstained sections were microwaved for 30 sec in citrate buffer before incubation for 4 hr at room temperature with the optimal dilution of antibody (2 μg/ml). A biotinolyated secondary antibody was applied for 30 min. Specific staining was revealed using a standard kit according to the manufacturer's directions (Biogenex). Normal human prostates were obtained from autopsy and were frozen at −80°C. Frozen sections were stained as described for formalin fixed tissue. Formalin fixed tissues were also used in the staining of human prostates. The specificity of sc-61 antibody for C/EBPα was confirmed using HeLa cells transfected with an expression plasmid for C/EBPα (data not shown). In all cases, a non-immune rabbit IgG (sc-2027, Santa Cruz Biotechnology, Inc.) (negative control) was non-reactive.

Isolation of RNA From Mouse and Human Prostate

Prostate tissues from mice of various age groups were dissected immediately following euthanasia of the mice and homogenized in TRIZOL Reagent (Invitrogen) using a TissueMiser (Fisher). Total RNA was isolated from the tissue homogenates following the manufacturer's protocol for TRIZOL Reagent. Total RNA was similarly isolated after pulverizing frozen human prostate tissue. Control adult human liver RNA was purchased from Ambion (Foster City, CA).

Real Time-ReverseTranscription-Polymerase Chain Reaction (Real Time-RT-PCR) Analysis

Reverse transcription followed by quantitative real-time PCR was used to measure the endogenous mRNA levels for mouse or human C/EBPα and for mouse or human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (control). Total RNA (1 μg per sample) was reverse transcribed with random primers using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to manufacturers' protocol. The reverse transcription product was subjected to quantitative real-time PCR using the Real-Time PCR master mix (Applied Biosystems) in the 7500 Real-Time PCR System (Applied Biosystems). Primers and TaqMan probe for mouse and human C/EBPα and for human GAPDH were obtained from Applied Biosystems. The mRNA for mouse GAPDH was measured using the Syber Green I nucleic acid gel stain from Molecular Probes (Invitrogen detection technologies). Fluorescence data generated were monitored and recorded on a 7500 Real-Time PCR sequence detection system (Applied Biosystems). All samples were measured in triplicate and the values normalized to the corresponding GAPDH values. As a negative control, the Real Time PCR master mix was used without any template added during the reverse transcription step of the quantitative real time RT-PCR assays. The results are presented as mean ± SE.

Tissue Array Analysis

Replicate prostate normal and cancer tissue array slides (#PR802, #PR952, and #BC19111) were purchased from US Biomax, Inc. (Rockville, MD). The arrays were probed with primary rabbit antibody to

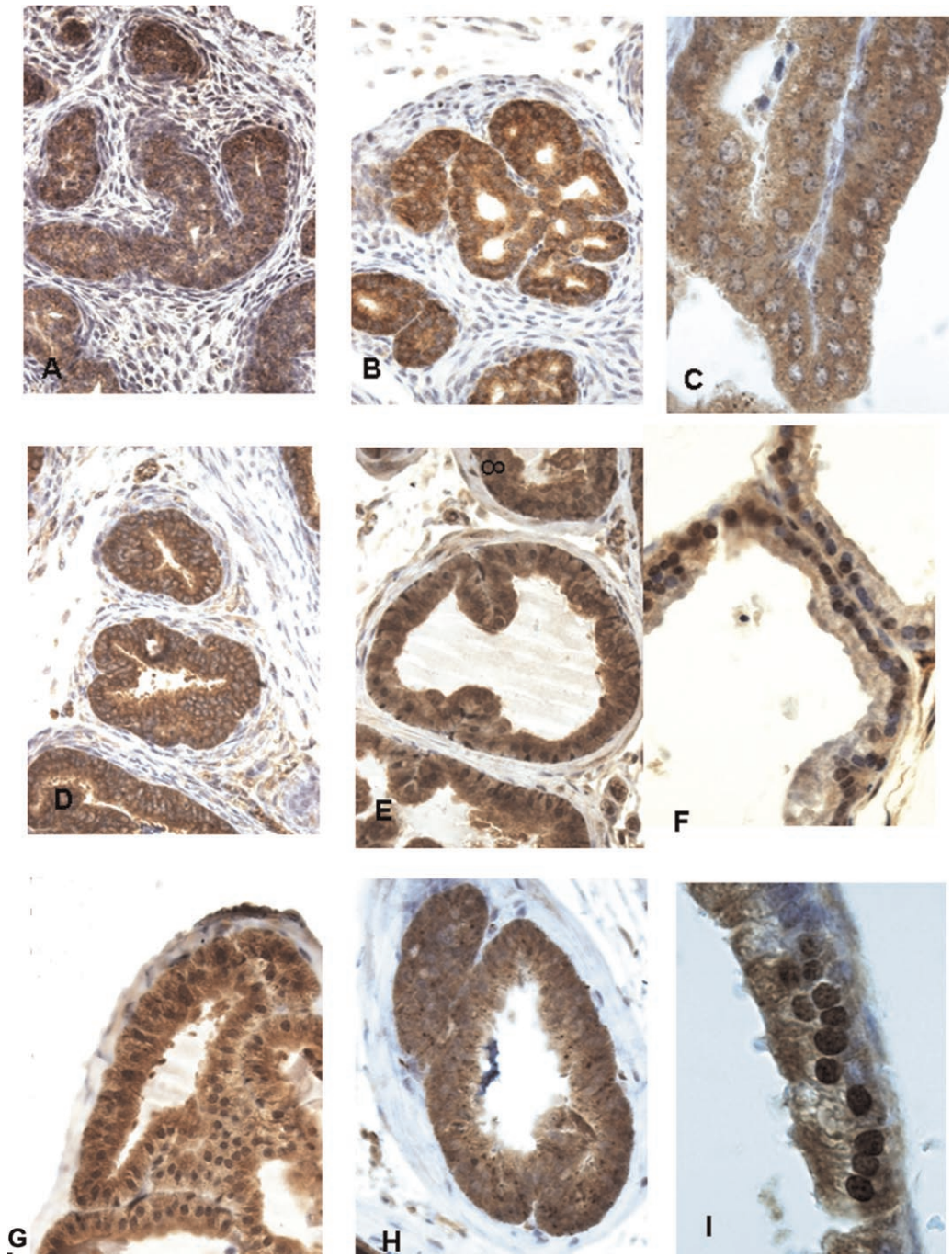

Fig. 1. Expression and localization of C/EBPα in the mouse prostate epithelium during development. **A**: Uniform staining of the cytoplasm of the prostate epithelium already present at 1 week of age. **B**: Diffuse staining of the cytoplasm of the prostate epithelium at 3 weeks of age. **C**: Higher magnification of the prostate of a 3-week-old mouse. Note the complete lack of nuclear staining. **D**: Diffuse staining of the cytoplasm only in the epithelium still present at 6 weeks. **E**: Diffuse staining of the prostatic epithelium including some nuclear staining at 8 weeks. **F**: Diffuse cytoplasmic and prominent nuclear staining in the prostatic epithelium of a 10-week-old mouse. **G**: Diffuse cytoplasmic and nuclear staining at 16 weeks of age. **H**: Uniform staining of the cytoplasm and nuclei in 8-month-old mouse. **I**: Higher magnification of prostate of an 8-month-old mouse demonstrates cytoplasmic and prominent nuclear staining in the prostatic epithelium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

either C/EBPα (SC-61, Santa Cruz Biotechnology, Inc.) or to AR (SC-816, Santa Cruz Biotechnology, Inc.) or with normal rabbit IgG (negative control). Antigen retrieval was performed using Target Retrieval Solution (S1699) from DAKO. Sections were incubated for 30 min with ready-to-use (2.5%) normal horse blocking serum. They were then incubated with primary antibodies (2 μg/ml) for 1h at room temperature. After washing, the slides were incubated for 30 min with ImPRESS Reagent (peroxidase conjugated anti-rabbit IgG, MP7407 from Vector Laboratories). The sections were then washed and incubated for 5 min with peroxidase substrate (DAB, K3466 from DAKO Cytomation). After rinsing with tap water, the slides were counterstained with Hematoxilin QS (H-3404, Vector Laboratories). A duplicate set of slides were treated as above but without counterstaining. Twenty times object images were scanned for each slide.

RESULTS

Expression of C/EBPα During the Development of the Mouse Prostate

The temporal sequence of C/EBPα expression in the developing mouse prostate was examined by immunohistochemical staining (Fig. 1). C/EBPα was expressed throughout the cytoplasm of the prostate epithelial cells as early as 1 week; cytoplasmic expression remained strong and diffuse up to 6 weeks of age. At 8 weeks of age, C/EBPα was expressed diffusely in both the cytoplasm and in most nuclei of the prostate epithelium; strong, uniform expression of C/EBPα in both the cytoplasm and nuclei of the prostate epithelium persisted at 16 weeks. By 8 months, expression of C/EBPα was still diffuse in the cytoplasm and prominent but not complete in the nuclei. The expression of C/EBPα in the liver of the adult mouse remained relatively high and was also nuclear (data not shown).

The mRNA level for C/EBPα remained relatively constant throughout the development of the mouse prostate (1–12 weeks) and was significantly lower (~1/30) compared to the level of C/EBPα mRNA in the adult mouse liver (Fig. 2); this may be at least partially accounted for by the fact that there is a greater proportion of C/EBPα-negative interstitial tissue in the prostate. The C/EBPα mRNA decreased modestly in older (6 months and over) mice (Fig. 2).

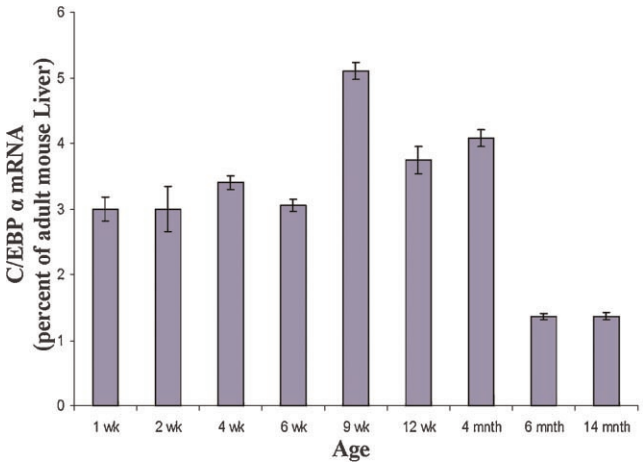

Fig. 2. Quantitative analysis of C/EBPα mRNA expression in the developing mouse prostate. The amount of mRNA for mouse C/EBPα was determined from at least two mice from each age group by real time RT-PCR and normalized to the corresponding value for GAPDH. The values are represented as the fraction of C/EBPα mRNA from an adult mouse liver. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Expression of C/EBPα in the Normal Human Prostate

Figure 3 shows representative examples of the expression of C/EBPα in the normal human prostate.

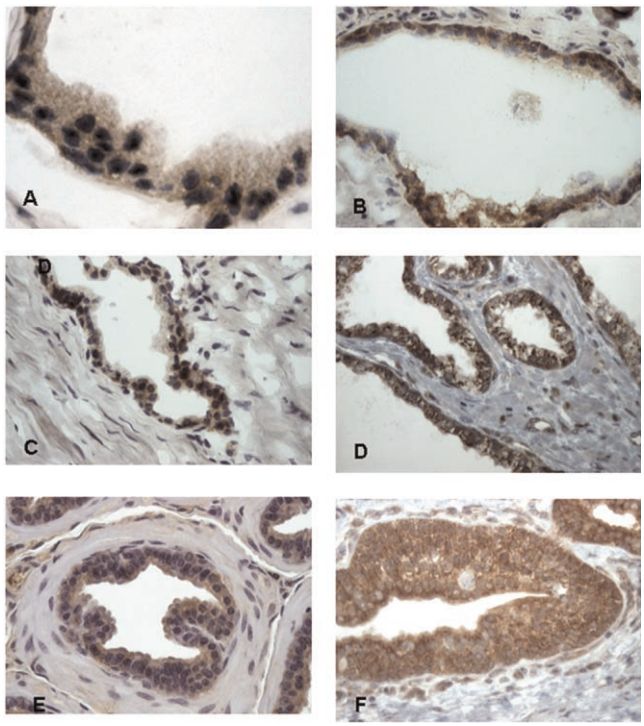

Fig. 3. Expression and localization of C/EBPα in the human prostate epithelium in relation to age. **A**: Diffuse nuclear and cytoplasmic staining of the prostate epithelium at 16 years of age. Intense nuclear and cytoplasmic staining is maintained at 27 (**B**) and 29 (**C**) years of age (**D**) Prominent but not diffuse expression of C/EBPα in the nucleus and diffuse staining in the cytoplasm of prostate epithelium in a 49-year-old male. **E**: Consistent cytoplasmic expression and occasional nuclear staining for C/EBPα at 51 years of age. **F**: C/EBPα is present in the cytoplasm but not in the nuclei of a 59-year-old man. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Subcellular Localization of C/EBPα in the Prostate Epithelia of Older Men

Sample No.	Age (years)	Sub-cellular localization
1	52	Focal nuclear/cytosolic
2	52	Focal nuclear/cytosolic
3	52	Focal nuclear/cytosolic
4	62	Cytosolic
5	63	Cytosolic/focal nuclear
6	63	Cytosolic
7	63	Cytosolic
8	63	Cytosolic
9	63	Cytosolic
10	65	Cytosolic
11	65	Cytosolic
12	68	Cytosolic
13	69	Cytosolic/focal nuclear
14	70	Cytosolic
15	70	Cytosolic
16	70	Cytosolic
17	77	Cytosolic/focal nuclear
18	77	Cytosolic/focal nuclear
19	77	Cytosolic

There was strong diffuse staining of C/EBPα in the nucleus and cytoplasm of prostate epithelial cells at 16 years of age (peri-pubescent). Nuclear and cytoplasmic expression of C/EBPα remained unchanged at 27 and 29 years of age. By 49 years of age, there was some decline in nuclear expression of C/EBPα. Loss of nuclear staining became widespread by age 59, with C/EBPα expression observed in the cytoplasm. Cytosolic localization of C/EBPα in the prostates of older men, noted in Figure 3, was further established by data that included tissue array analysis of normal prostate (Table I).

The mRNA for C/EBPα was expressed in human prostate at a relatively higher level than mouse prostate, when measured in relation to liver (~30% of liver C/EBPα mRNA) (Fig. 4); again, it may be noted that like the mouse prostate, the human prostate has a greater proportion of C/EBPα-negative interstitial tissue than liver.

Expression of C/EBPα in Prostate Adenocarcinoma

The expression of C/EBPα in prostate adenocarcinoma was examined together with that of AR by immunohistochemical analysis of a tumor tissue array containing diseased tissue from 78 patients. The patients ranged in age from 38 to 87 years and the tumor samples covered the entire range of tumor grade and Gleason scores. The signal intensities for both C/EBPα and AR were scored in arbitrary units in the range of 0 (absent) through 4 (highest). Representative results

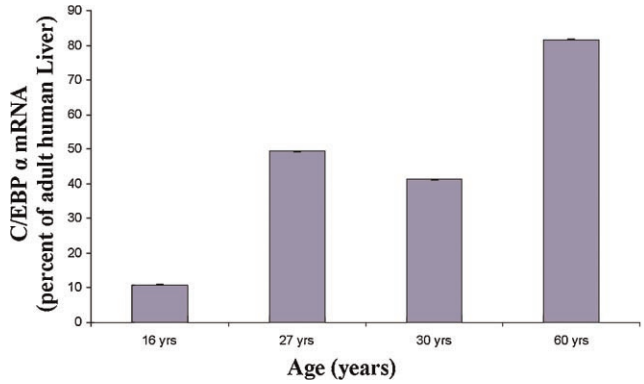

Fig. 4. Quantitative analysis of C/EBPα mRNA expression in the human prostate in relation to age. The amount of mRNA for human C/EBPα was determined by real time RT-PCR and normalized to the corresponding value for GAPDH. The values are represented as the fraction of C/EBPα mRNA from an adult human liver. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of for the staining of C/EBPα and AR together with negative controls are shown in Figure 5. C/EBPα expression in the tumors was predominantly in the cytosol although some cells in the sections showed nuclear staining. A box plot analysis of the results showed that samples with higher AR scores (3 and 4) co-expressed significantly higher levels of C/EBPα (Fig. 6A). However, there was no significant correlation between the Gleason score and the expression level of either C/EBPα or AR (data plot not shown).

To examine the relative mRNA levels for C/EBPα and AR in prostate adenocarcinoma samples from a large number of patients, DNA microarray data was mined from the largest single study of prostate tumors [27] through the Oncomine repository at: http://www.oncomine.org/ and also through the public database at: http://pcabc.upmc.edu/data/pubviewdata.cfm?cc=pitt&acctype=pub&proj=director%27s%20challenge%20for%20the%20molecular%20reclassification%20of%20prostate%20cancer. The box plot in Figure 6B shows an elevation in the mRNA for C/EBPα in prostate tumors relative to normal prostate. The box plot in Figure 6C indicates a significant elevation in AR mRNA in the more aggressive prostate tumors compared to non-aggressive tumors. Among the aggressive tumors, there was a significant positive correlation between the expression level of AR and that of C/EBPα ($r = 0.5111471$) (Fig. 6D), consistent with immunohistochemical studies of tumor tissue arrays discussed above.

DISCUSSION

C/EBPα gene knockout mice have been reported to die within hours after birth from hypoglycemia due to

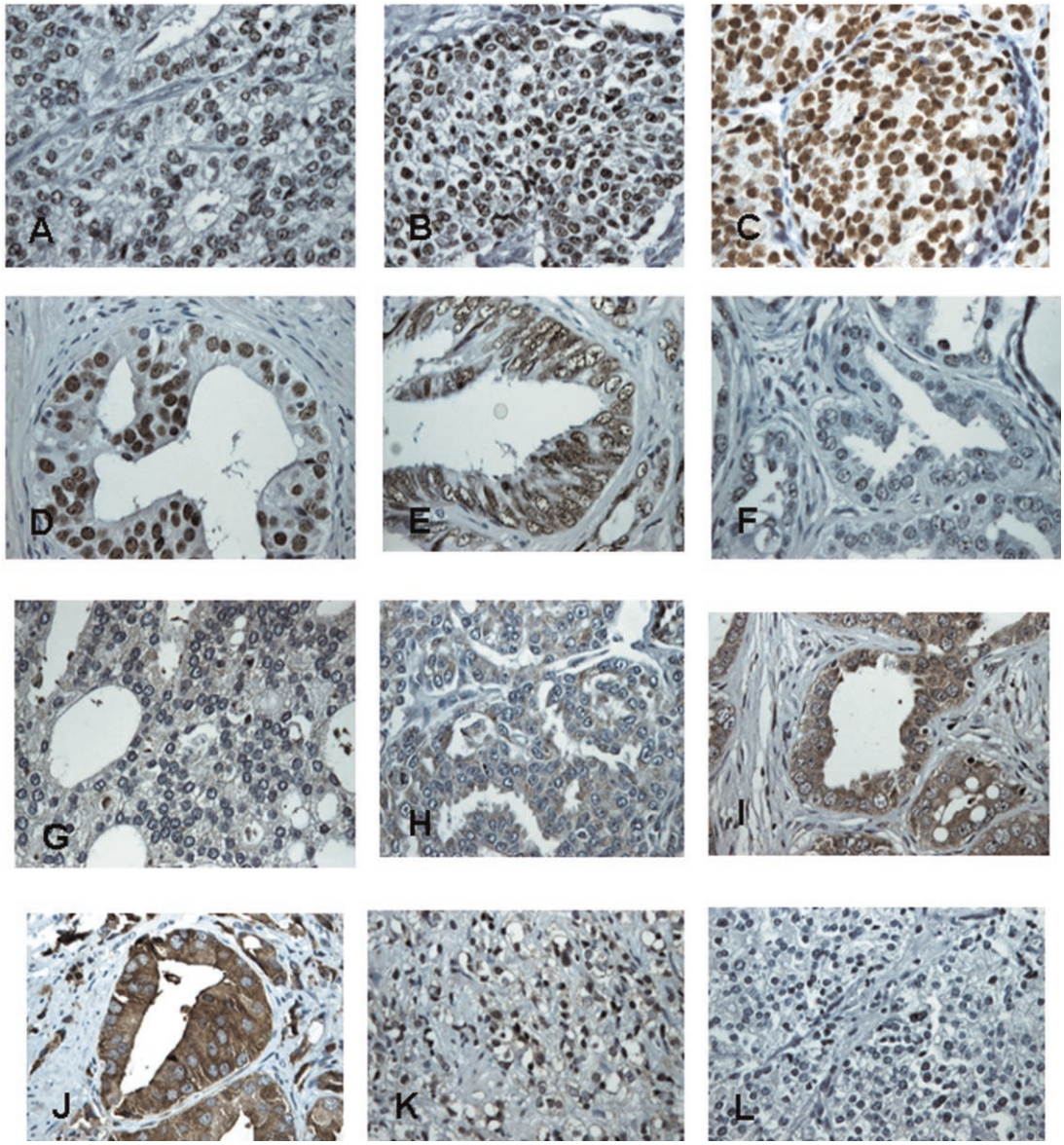

Fig. 5. Scoring of AR and C/EBPα expression in human prostate tumors. **A**: +1 nuclear expression of AR. **B**: +2 nuclear expression of AR. **C**: +3 nuclear expression of AR. **D**: +4 nuclear expression of AR. **E**: +2 nuclear and cytoplasmic expression of AR. **F**: No expression of AR. **G**: +1 cytoplasmic expression of C/EBPα. **H**: +2 cytoplasmic expression of C/EBPα. **I**: +3 cytoplasmic expression of C/EBPα. **J**: +4 cytoplasmic expression of C/EBPα. **K**: +2 nuclear and cytoplasmic expression of C/EBPα. **L**: No expression of C/EBPα. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

decreases in glycogen synthase and gluconeogenic enzymes in the liver [28]. This period precedes the time of maturation of the prostate but the expression of C/EBPα in the prostate suggests a role for it in prostate physiology. This study reveals features of C/EBPα expression that are distinctive in the normal human prostate in comparison to other normal human tissues or to the normal mouse prostate. The mouse prostate shows expression of C/EBPα protein and mRNA expression very early in its development but the protein is localized in the nuclear compartment, which is the site of its action, only at the onset of terminal differentiation; C/EBPα expression persists in the nuclear compartment in older mice. In the human prostate also, C/EBPα protein and mRNA expression was observed at all ages that were examined (from peri-pubescence through the 8th decade); however the protein was progressively sequestered in the cytosolic compartment in older men, in contrast to observations in the liver of older men [29] and adipose tissue in post-menopausal women [24], where C/EBPα occurred in the nucleus. It thus appears that in normal

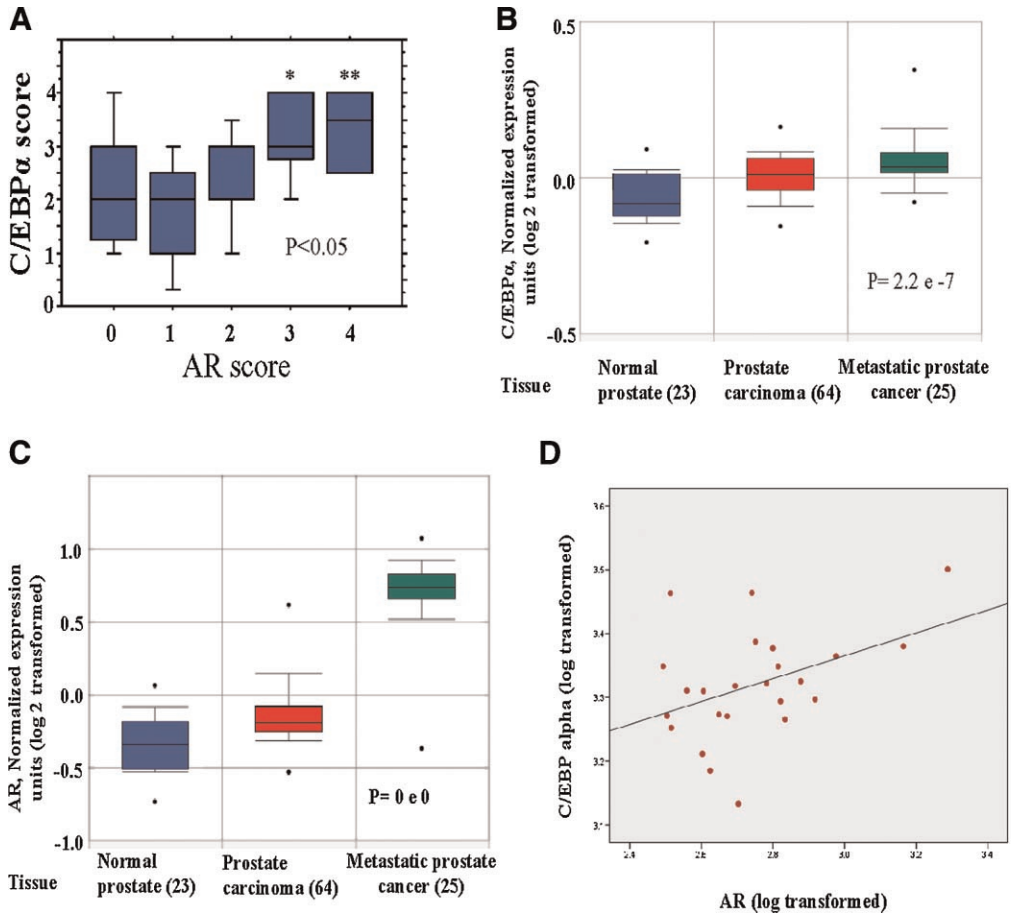

Fig. 6. Expression of C/EBPα and AR protein and mRNA in prostate tumors. **Panel A**: Immunohistochemical analysis of a prostate tumor tissue array for co-expression of AR and C/EBPα; scores in arbitrary units for the expression of C/EBPα and AR in a microarray containing 78 tumor specimens, determined as described under Materials and Methods Section, are graphed as a box plot. **Panel B**: Box plot of the expression of C/EBPα mRNA in prostate tumors retrieved from the Oncomine DNA microarray database. **Panel C**: Box plot of the expression of AR mRNA in prostate tumors retrieved from the same Oncomine DNA microarray data set as in panel B. **Panel D**: Scatter plot for the co-expression of C/EBPα and AR mRNAs represented in the metastatic prostate tumor group in Panels B and C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

prostate tissues, C/EBPα is regulated through modulation of its intracellular localization. The temporal sequence of expression and nuclear import of C/EBPα is entirely consistent with a role in prostate differentiation since the nucleus, rather than the cytosol, is the known site of most if not all of the biological actions of C/EBPα, particularly its strong antiproliferative actions. It is also reasonable to suggest then, that the age-related sequestration of the protein in the cytosol of the prostate epithelium may contribute significantly to the permissive state of the human prostate to the relatively high frequency of proliferative disorders in older males [30].

In contrast to other tumor suppressors, C/EBPα was frequently highly expressed in prostate tumors. Here again, the largely cytosolic localization of the protein would account for its inability to exert its antiproliferative effect. A similar, largely cytosolic localization was reported for C/EBPα in epithelial ovarian tumors [24]. In malignant tissues, however, the continued expression of C/EBPα may reflect a long-term role for the protein. Notably, a small fraction of the tumor cells did show the protein localized in the nucleus at any given time, indicating that some of the transcriptional activities of the protein may be utilized by the proliferating cells at the appropriate times by regulating its nuclear-cytoplasmic shuttling. Indeed nuclear-cytoplasmic shuttling of major transcription factors, including members of the C/EBP family, is known to be regulated through signaling pathways [31,32]. The specific proliferative versus antiproliferative role of C/EBPα may be controlled by regulating nuclear-cytoplasmic transport in relation to cellular contexts such as the cell cycle stage. In this regard, a significant observation is the frequent co-expression of C/EBPα with AR in prostate cancer, particularly in the

more aggressive/metastatic tumors. AR signaling is critical for the physiology of most prostate tumors even when the tumors are hormone refractory [33]. It has recently been reported that C/EBPα can physically associate with AR and influence gene transcription by AR, although the impact of this interaction on global gene expression profiles is yet to be analyzed [26]. The co-expression of the two interacting proteins, which are both major regulators of transcription, strongly suggests a combined role for them in determining the molecular phenotype of prostate tumors.

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REFERENCES

1. Ramji DP, Foka P. CCAAT/enhancer-binding proteins: Structure, function and regulation. Biochem J 2002;365(Pt 3):561–575.
2. Osada S, Yamamoto H, Nishihara T, Imagawa M. DNA binding specificity of the CCAAT/enhancer-binding protein transcription factor family. J Biol Chem 1996;271(7):3891–3896.
3. Umek RM, Friedman AD, McKnight SL. CCAAT-enhancer binding protein: A component of a differentiation switch. Science 1991;251(4991):288–292.
4. Hendricks-Taylor LR, Darlington GJ. Inhibition of cell proliferation by C/EBP alpha occurs in many cell types, does not require the presence of p53 or Rb, and is not affected by large T-antigen. Nucleic Acids Res 1995;23(22):4726–4733.
5. Watkins PJ, Condreay JP, Huber BE, Jacobs SJ, Adams DJ. Impaired proliferation and tumorigenicity induced by CCAAT/enhancer-binding protein. Cancer Res 1996;56(5):1063–1067.
6. Harris TE, Albrecht JH, Nakanishi M, Darlington GJ. CCAAT/enhancer-binding protein-alpha cooperates with p21 to inhibit cyclin-dependent kinase-2 activity and induces growth arrest independent of DNA binding. J Biol Chem 2001;276(31):29200–29209.
7. Timchenko NA, Wilde M, Nakanishi M, Smith JR, Darlington GJ. CCAAT/enhancer-binding protein alpha (C/EBP alpha) inhibits cell proliferation through the p21 (WAF-1/CIP-1/SDI-1) protein. Genes Dev 1996;10(7):804–815.
8. Timchenko NA, Harris TE, Wilde M, Bilyeu TA, Burgess-Beusse BL, Finegold MJ, Darlington GJ. CCAAT/enhancer binding protein alpha regulates p21 protein and hepatocyte proliferation in newborn mice. Mol Cell Biol 1997;17(12):7353–7361.
9. Timchenko NA, Wilde M, Iakova P, Albrecht JH, Darlington GJ. E2F/p107 and E2F/p130 complexes are regulated by C/EBPalpha in 3T3-L1 adipocytes. Nucleic Acids Res 1999;27(17):3621–3630.
10. Timchenko NA, Wilde M, Darlington GJ. C/EBPalpha regulates formation of S-phase-specific E2F-p107 complexes in livers of newborn mice. Mol Cell Biol 1999;19(4):2936–2945.
11. Porse BT, Pedersen TA, Xu X, Lindberg B, Wewer UM, Friis-Hansen L, Nerlov C. E2F repression by C/EBPalpha is required for adipogenesis and granulopoiesis in vivo. Cell 2001;107(2):247–258.
12. Wang H, Iakova P, Wilde M, Welm A, Goode T, Roesler WJ, Timchenko NA. C/EBPalpha arrests cell proliferation through direct inhibition of Cdk2 and Cdk4. Mol Cell 2001;8(4):817–828.
13. Wang H, Goode T, Iakova P, Albrecht JH, Timchenko NA. C/EBPalpha triggers proteasome-dependent degradation of cdk4 during growth arrest. EMBO J 2002;21(5):930–941.
14. Muller C, Calkhoven CF, Sha X, Leutz A. The CCAAT enhancer-binding protein alpha (C/EBPalpha) requires a SWI/SNF complex for proliferation arrest. J Biol Chem 2004;279(8):7353–7358.
15. Pabst T, Mueller BU, Harakawa N, Schoch C, Haferlach T, Behre G, Hiddemann W, Zhang DE, Tenen DG. AML1-ETO downregulates the granulocytic differentiation factor C/EBPalpha in t(8;21) myeloid leukemia. Nat Med 2001;7(4):444–451.
16. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, Behre G, Hiddemann W, Tenen DG. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet 2001;27(3):263–270.
17. Burel SA, Harakawa N, Zhou L, Pabst T, Tenen DG, Zhang DE. Dichotomy of AML1-ETO functions: Growth arrest versus block of differentiation. Mol Cell Biol 2001;21(16):5577–5590.
18. Halmos B, Huettner CS, Kocher O, Ferenczi K, Karp DD, Tenen DG. Down-regulation and antiproliferative role of C/EBPalpha in lung cancer. Cancer Res 2002;62(2):528–534.
19. Gery S, Tanosaki S, Bose S, Bose N, Vadgama J, Koeffler HP. Down-regulation and growth inhibitory role of C/EBPalpha in breast cancer. Clin Cancer Res 2005;11(9):3184–3190.
20. Loomis KD, Zhu S, Yoon K, Johnson PF, Smart RC. Genetic ablation of CCAAT/enhancer binding protein alpha in epidermis reveals its role in suppression of epithelial tumorigenesis. Cancer Res 2007;67(14):6768–6776.
21. Schuster MB, Porse BT. C/EBPalpha: A tumour suppressor in multiple tissues? Biochim Biophys Acta 2006;1766(1):88–103.
22. Wang GL, Iakova P, Wilde M, Awad S, Timchenko NA. Liver tumors escape negative control of proliferation via PI3K/Akt-mediated block of C/EBP alpha growth inhibitory activity. Genes Dev 2004;18(8):912–925.
23. Wang GL, Timchenko NA. Dephosphorylated C/EBPalpha accelerates cell proliferation through sequestering retinoblastoma protein. Mol Cell Biol 2005;25(4):1325–1338.
24. Sundfeldt K, Ivarsson K, Carlsson M, Enerback S, Janson PO, Brannstrom M, Hedin L. The expression of CCAAT/enhancer binding protein (C/EBP) in the human ovary in vivo: Specific increase in C/EBPbeta during epithelial tumour progression. Br J Cancer 1999;79(7–8):1240–1248.
25. Yin H, Radomska HS, Tenen DG, Glass J. Down regulation of PSA by C/EBPalpha is associated with loss of AR expression and inhibition of PSA promoter activity in the LNCaP cell line. BMC Cancer 2006;6:158.
26. Chattopadhyay S, Gong EY, Hwang M, Park E, Lee HJ, Hong CY, Choi HS, Cheong JH, Kwon HB, Lee K. The CCAAT enhancer-binding protein-alpha negatively regulates the transactivation of androgen receptor in prostate cancer cells. Mol Endocrinol 2006;20(5):984–995.

27. Yu YP, Landsittel D, Jing L, Nelson J, Ren B, Liu L, McDonald C, Thomas R, Dhir R, Finkelstein S, Michalopoulos G, Becich M, Luo JH. Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. J Clin Oncol 2004;22(14):2790–2799.
28. Wang ND, Finegold MJ, Bradley A, Ou CN, Abdelsayed SV, Wilde MD, Taylor LR, Wilson DR, Darlington GJ. Impaired energy homeostasis in C/EBP alpha knockout mice. Science 1995;269(5227):1108–1112.
29. Xu LX, Sui YF, Wang WL, Liu YF, Gu JR. Immunohistochemical demonstration of CCAAT/enhancer binding protein (C/EBP) in human liver tissues of various origin. Chin Med J (Engl) 1994;107(8):596–599.
30. Brown TR, Lee C. Conference summary on prostate growth and aging, 13–15 September 2000. Prostate 2001;48(1):54–65.
31. Ghosh S, Baltimore D. Activation in vitro of NF-kappa B by phosphorylation of its inhibitor I kappa B. Nature 1990; 344(6267):678–682.
32. Yin M, Yang SQ, Lin HZ, Lane MD, Chatterjee S, Diehl AM. Tumor necrosis factor alpha promotes nuclear localization of cytokine-inducible CCAAT/enhancer binding protein isoforms in hepatocytes. J Biol Chem 1996;271(30):17974–17978.
33. Zegarra-Moro OL, Schmidt LJ, Huang H, Tindall DJ. Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. Cancer Res 2002;62(4):1008–1013.