ARTICLE
Journal of Cellular Biochemistry 112:2742–2747 (2011)

Journal of Cellular Biochemistry

Genome-Wide Mapping of ZNF652 Promoter Binding Sites in Breast Cancer Cells

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ABSTRACT

A significant proportion of transcription factors encoded by the human genome are classical C_2H_2 zinc finger proteins that regulate gene expression by directly interacting with their cognate DNA binding motifs. We previously showed that one such C_2H_2 zinc finger DNA binding protein, ZNF652 (zinc finger protein 652), specifically and functionally interacts with CBFA2T3 to repress transcription of genes involved in breast oncogenesis. To identify potential targets by which ZNF652 exerts its putative tumour suppressive function, its promoter-specific cistrome was mapped by ChIP-chip. De novo motif scanning of the ZNF652 binding sites identified a novel ZNF652 recognition motif that closely resembles the previously characterised in vitro binding site, being a 10 nucleotide core of that 13 nucleotide sequence. Genes with ZNF652 binding sites function in diverse cellular pathways, and many are involved in cancer development and progression. Characterisation of the in vivo ZNF652 DNA binding motif and identification of potential ZNF652 target genes are key steps towards elucidating the function(s) of this transcription factor in the normal and malignant breast cell. J. Cell. Biochem. 112: 2742–2747, 2011.

KEY WORDS: ZNF652; CBFA2T3; TRANSCRIPTION FACTOR; ChIP-chip

Transcriptional regulation by RNA polymerase II is critical during all stages of the development and differentiation of higher organisms [Park, 2009]. Such regulation occurs primarily through a large and diverse group of proteins, termed transcription factors, that are encoded by approximately 10% of the genes in the human genome [Kummerfeld and Teichmann, 2006]. Classical C_2H_2 zinc finger transcription factors are a highly conserved family of sequence-specific DNA binding proteins and the largest group of transcription factors, comprising 675 of the 1,900 encoded in the human genome [Vaquerizas et al., 2009]. Given their ubiquity, it is not surprising that zinc finger transcription factors play critical roles in a wide range of cellular regulatory pathways [Vaquerizas et al., 2009].

The C_2H_2 zinc finger transcription factor ZNF652 was identified as a specific DNA-binding partner of the Eight-Twenty One (ETO) protein CBFA2T3 from a breast expression library in a yeast two-hybrid screen [Kumar et al., 2006]. We previously showed that ZNF652 expression is high in the normal mammary tissue but is reduced in breast tumours [Kumar et al., 2006, 2010]. Furthermore, we found that ZNF652 specifically and functionally interacts with CBFA2T3 to repress transcription of genes that function in breast oncogenesis [Kumar et al., 2006]. These results, along with the location of *ZNF652* on the long arm of chromosome 17q, a region of frequent loss of heterozygosity in cancer, suggested that this gene may also have a role in development of other malignancies.

Subsequently, in an effort to discover transcriptional targets of ZNF652, we identified a DNA motif recognised by ZNF652 in vitro [Kumar et al., 2008] and found that a related transcription factor, ZNF651, also binds to this DNA sequence [Kumar et al., 2010]. In addition, using an in silico approach, we demonstrated that the *HEB* gene promoter contains a functional ZNF652 DNA binding motif and that expression of *HEB* is regulated by the CBFA2T3–ZNF652 repressor complex [Kumar et al., 2008].

R. Kumar and L.A. Selth contributed equally to this work.

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: National Breast Cancer Foundation, Australia.

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Received 19 January 2011; Accepted 1 June 2011 • DOI 10.1002/jcb.23214 • © 2011 Wiley-Liss, Inc.
Published online 15 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

Characterisation of ZNF652 DNA binding sites in vivo is likely to identify additional genes regulated by this transcription factor, some of which may represent new therapeutic targets for breast cancer. To isolate ZNF652-regulated genes and as a first step in defining the ZNF652 cistrome, we characterised the genome-wide promoter binding profile of this transcription factor using ChIP-chip. Bioinformatic analyses of these binding sites revealed a novel ZNF652 DNA binding motif and identified many genes and diverse cellular pathways that are potentially under direct transcriptional control of ZNF652. These findings will help to determine how ZNF652-mediated transcriptional repression suppresses oncogenesis in the normal cell and how disruption of its function may result in carcinogenesis.

EXPERIMENTAL PROCEDURES

CELL CULTURE AND CHIP-CHIP ASSAY

ZR75-1 human breast cancer cells were grown in RPMI1640 medium (Invitrogen) supplemented with 1% penicillin/streptomycin/L-glutamine, 1 mM sodium pyruvate and 10% foetal bovine serum and incubated at 37°C in a humidified 5% CO_2 incubator. Approximately 2×10^7 cells in suspension were cross-linked with 1% formaldehyde solution for 9 min at room temperature on a rotary mixer. The reaction was stopped with cold 0.125 M glycine and cells were washed twice with cold 1× PBS. Cells were lysed with lysis buffer (50 mM Tris–HCl pH 8.0, 10 mM EDTA, 1% SDS) supplemented with protease inhibitors (Roche) for 10 min and then sonicated on a Vibra-Cell VCX 130 (Sonics) using a 3-mm tip with six 15-s pulses at 30% amplitude. Clarified lysates were diluted with 10 volumes of ChIP dilution buffer (16.7 mM Tris–HCl pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100) containing protease inhibitors (Roche) and incubated with affinity-purified rabbit anti-ZNF652 or anti-RNA polymerase II antibody N-20 (sc-899X, Santa Cruz Biotechnology) overnight at 4°C. The following day, control or anti-ZNF652-chromatin immunocomplexes were harvested by adding Protein A beads that had been pre-incubated with 400 μg/ml sonicated salmon sperm DNA (Sigma) and 500 μg/ml BSA in 10 mM Tris–HCl pH 8.0, 1 mM EDTA. Beads were sequentially washed with low salt wash buffer (20 mM Tris–HCl pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), high salt wash buffer (20 mM Tris–HCl pH 8.0, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), LiCl immune complex wash buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA, 0.25 M LiCl, 1% NP40, 1% deoxycholate) and finally TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA). A fraction of the protein–DNA complexes were eluted with SDS protein-loading buffer (62.5 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol) at 95°C for 5 min for Western blot analysis with the anti-ZNF652 antibody. The remainder of the immunoprecipitated material was eluted in 250 μl elution buffer (1% SDS, 0.1 M $NaHCO_3$) and cross-links were reversed by addition of 10 μl 5 M NaCl and heating at 65°C for 4 h. This mixture was then supplemented with 10 μl 0.5 M EDTA, 20 μl 1 M Tris–HCl pH 6.5 and 2 μl of 10 mg/ml Proteinase K and incubated at 45°C for 1 h. Input and anti-ZNF652-immunoprecipitated DNA were purified using MinElute PCR Purification Kit (Qiagen) and amplified by GenomePlex complete whole genome amplification (WGA) kit (Sigma) according to the manufacturer's protocol. The amplified DNAs were again purified using MinElute PCR Purification Kit, assayed and hybridised to the HG18 RefSeq promoter tiling microarray (NimbleGen Systems, Inc.). This array contains approximately 19,000 well-characterised RefSeq genes tiled from −2200 to +500 bp around the transcriptional start site (TSS). ZNF652 binding sites (peaks) were identified using NimbleScan (false discovery rate ≤ 0.2).

CHIP-qPCR

Three ZNF652 gene targets (*PUS10*, *GRHL3* and *BGLAP*) identified from the ChIP-chip analysis were selected for validation by ChIP-quantitative PCR (qPCR). ChIP was performed on ZR75-1 and normal mammary epithelial MCF10A cells as described above. The immunoprecipitated DNA was used as template in qPCR reactions. qPCRs were performed using iQ SYBR Green Supermix (Bio-Rad) and gene-specific primers under the following conditions: 95°C for 30 s; 40 cycles of 94°C for 15 s, 55°C for 15 s and 72°C for 30 s. A region 10 kb downstream of the 3′-UTR of *CDC25B* was used as a negative control. Primer sequences are shown in Table I.

MOTIF SEARCHING AND ANALYSIS

Gibbs Motif Sampler [Thompson et al., 2003] was used to isolate enriched de novo motifs within these sites. Fold enrichment and significance (calculated using Fisher's exact test) of novel motifs were estimated by comparisons with 1,130 1 kb control regions of matched physical distribution [Ji et al., 2006, 2008]. A conservation profile of ZNF652 motif sequences was generated using the Cistrome Analysis Pipeline, which uses PhastCons conservation scores based on multiz alignment of human, chimpanzee, mouse, rat, dog, chicken, fugu and zebrafish genomic DNA. To scan for known motifs in the ZNF652 binding sites, position weight matrices representing transcription factor binding motifs were obtained from the JASPAR CORE vertebrata database [Bryne et al., 2008]. CisGenome [Ji et al., 2008] was used to identify matches to these matrices (likelihood ratio ≥ 500). This software was also used to scan the entire genome for the ZNF652 DNA binding sequence without allowing any mismatches.

RESULTS

ANTI-ZNF652 ANTIBODY SPECIFICALLY AND EFFICIENTLY IMMUNOPRECIPITATES ZNF652-CHROMATIN COMPLEXES

We have previously shown that rabbit anti-ZNF652 antibody specifically detects the endogenous ZNF652 protein from ZR75-1

TABLE I. List of Oligonucleotides Used in ChIP-qPCR

Gene	Orientation	Primer sequence (5′ → 3′)	Product size (bp)
PUS10	F	CAAGCAAGCCCTTCCCCCTTCG	279
	R	AACGCACGTTTCCTCGGGGAAC	
BGLAP	F	TCTCAAGCCCTGGTTGGTGGGT	114
	R	ACACCTGAGAGCCTCCACTGCC	
GRHL3	F	GCCGCCGCCGTTCTATCTGATC	261
	R	CCGCCGTTAGGGTTGGCTCAAG	
CDC25B	F	GCCCCTCTAGGTAGGTGGGCTG	173
	R	CACCCAGTGGCTGCAGATGTGG	

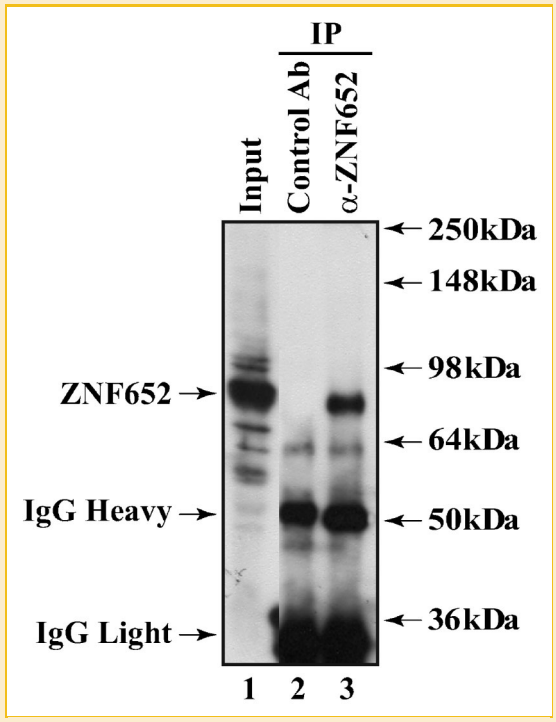

Fig. 1. Anti-ZNF652 antibody specifically and efficiently immunoprecipitates cross-linked chromatin–DNA complexes. Sheared chromatin generated from nuclear extracts of formaldehyde-treated asynchronous ZR75-1 cells was immunoprecipitated with anti-RNA polymerase II (negative control) or anti-ZNF652 antibodies. Input (lane 1; equivalent to 1% of the immunoprecipitated samples in lane 2–3) and immunoprecipitated complexes (lane 2–3) were resolved by SDS–PAGE, transferred to a nitrocellulose membrane and Western blotted with the anti-ZNF652 antibody. Endogenous ZNF652 protein is shown by an arrow. No signal for RNA polymerase II (which migrates at ~240 kDa) was detected in lane 2.

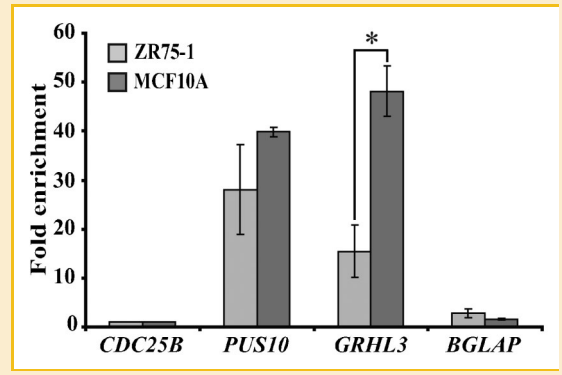

Fig. 2. Validation of ZNF652 gene targets in ZR75-1 and MCF10A cells. ChIP coupled to qPCR was used to measure the enrichment of ZNF652 at three putative binding sites within the promoters of *PUS10*, *GRHL3* and *BGLAP*. ChIP assays were performed separately with ZNF652 and control (IgG) antibodies. The level of ZNF652-specific chromatin enrichment compared to rabbit IgG is shown. A region downstream of the *CDC25B* gene that is not bound by ZNF652 was used as a negative control (set to 1). Experiments were performed in triplicate. Error bars represent ±standard error of the mean. Student's *t*-tests were used to compare enrichment of ZNF652 in ZR75-1 and MCF10A cells at specific loci ($^{*}P < 0.05$).

nuclear extracts [Kumar et al., 2006]. To determine whether this antibody was suitable for ChIP assays, chromatin–ZNF652 complexes were immunoprecipitated from formaldehyde cross-linked ZR75-1 nuclear extracts and analysed by Western blotting. An unrelated rabbit antibody that recognises RNA polymerase II was used as a negative control for the immunoprecipitation step (Fig. 1). The anti-ZNF652 antibody was able to specifically and efficiently immunoprecipitate the cross-linked endogenous ZNF652 protein, which was detected as a discrete 85 kDa band after Western blotting (Fig. 1). This indicated that cross-linking does not mask or otherwise perturb the anti-ZNF652 antibody binding epitope and that this antibody would be suitable for the ChIP assay.

CHARACTERISATION OF A PROMOTER-SPECIFIC ZNF652 CISTROME

To identify endogenous ZNF652 binding sites, we enriched for ZNF652-bound chromatin from asynchronous ZR75-1 cells using our in-house anti-ZNF652 antibody. ZR75-1 is a human breast cancer cell line known to express relatively high levels of ZNF652 [Kumar et al., 2006]. DNA isolated from the ZNF652-chromatin immunoprecipitates were hybridised to a NimbleGen promoter array containing ~19,000 promoters from well-characterised human genes. After correcting for input DNA signals, 113 promoters were found to contain one or more putative high-affinity ZNF652 DNA binding sites (Supplementary Table 1).

To validate the ChIP-chip data, we performed independent ChIP-qPCR for three of the binding sites located in the promoters of *PUS10*, *GRHL3* and *BGLAP*. A region 10 kb downstream of the *CDC25B* gene was used as a negative control. The *PUS10*, *GRHL3* and *BGLAP* binding sites exhibited approximately 28-, 15- and 3-fold enrichment in ZR75-1 cells, respectively, relative to the non-specific control chromatin region near *CDC25B* (Fig. 2). This result suggested that the ChIP-chip data were robust and that these genes are *bona fide* transcriptional targets of ZNF652. We also tested whether ZNF652 binds to these sites in MCF10A cells, a model of non-malignant breast epithelium. ZNF652 exhibited a similar affinity for the *PUS10* and *BGLAP* promoters in ZR75-1 and MCF10A cells but showed significantly higher enrichment at the *GRHL3* promoter in MCF10A cells (Fig. 2).

IDENTIFICATION OF A NOVEL ZNF652 DNA BINDING MOTIF

We previously identified CGAAAGGGTTAAT as a consensus ZNF652 DNA binding sequence using an in vitro CASTing protocol and electrophoretic mobility shift assays [Kumar et al., 2008]. To determine whether similar sequences direct ZNF652 binding in vivo, the 113 promoter binding sites were analysed for enriched sequences that may represent de novo ZNF652 binding motifs using a Gibbs sampling approach (see the Experimental Procedures Section). The most highly enriched motif was a 10 bp sequence MAAGGGTTAA (where M = AC) that forms the core of the previously identified in vitro ZNF652 binding site (Fig. 3A). This sequence was centred within the identified binding sites (Fig. 4A) and highly conserved amongst vertebrates (Fig. 4B), demonstrating its functionality in directing ZNF652 binding and highlighting the robustness of the ChIP-chip data. Of the total 113 promoter binding sites, 41 (36%)

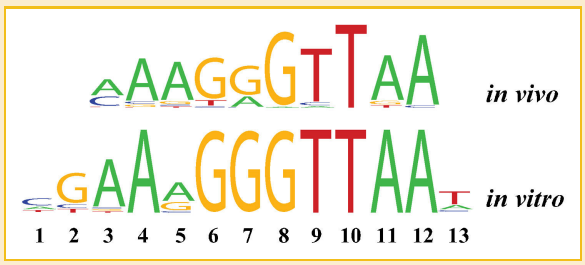

Fig. 3. Identification of an in vivo ZNF652 recognition motif. A Gibbs sampling approach was used to identify de novo motifs within the 113 ZNF652 promoter binding sites. Graphical representation (WebLogo; [Crooks et al., 2004]) of the in vivo motif (top) shows that it closely resembles the core region of the previously identified in vitro ZNF652 motif (bottom) [Kumar et al., 2008]. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

contained a sequence resembling this motif (Fig. 4C; Supplementary Table 1).

The promoter sequences identified from the array were also scanned for over-represented transcription factor binding motifs from the JASPAR database [Bryne et al., 2008]. The most highly enriched motifs included those bound by homeobox (i.e. Nobox, HOXA5, Pou5f1 and Lhx3) and forkhead (i.e. Foxq1, FOXC1, FOXD1 and FOXF2) factors (Table II). We separately scanned the promoter sequences which lacked the ZNF652 binding motif and found differential enrichment of several motifs including those recognised by the androgen and oestrogen receptors (AR and ER), CREB1 and NF-kappaB (Table II).

Recent genome-wide studies of transcription factors have revealed that cis-acting regulatory elements are frequently found in enhancer regions that may be far from the gene under transcriptional control [Carroll et al., 2006; Farnham, 2009]. To identify ZNF652 binding sites outside of promoters we performed a stringent full genome scan using the 10 bp consensus ZNF652 DNA binding sequence. Approximately 2,000 genes possess one or more ZNF652 DNA recognition sequences within 50 kb of the transcription start sites (TSS). Many of these genes (e.g. *BRCA1*, *ATM*, *KRAS*, *CHEK2* and *FOXO1*) have been implicated in the genesis of a wide range of malignancies, including breast cancer. However, given the relatively high occurrence of this motif throughout the genome, it is likely that genes with relevance to breast cancer will frequently have a proximal ZNF652 consensus site by chance alone. To extract more biologically relevant data from this gene set we used Ingenuity Pathway Analysis software to identify enriched pathways (Supplementary Table 2). This analysis revealed that genes classified in pathways that contribute to the development of breast cancer, including Wnt/β-catenin signalling, molecular mechanisms of cancer, ATM signalling, cyclins and cell cycle regulation and breast cancer regulation by stathmin1, are more likely to have a proximal ZNF652 consensus binding motif (Supplementary Table 2).

DISCUSSION

Transcriptional dysregulation due to aberrant transcription factor function is frequently associated with diverse developmental

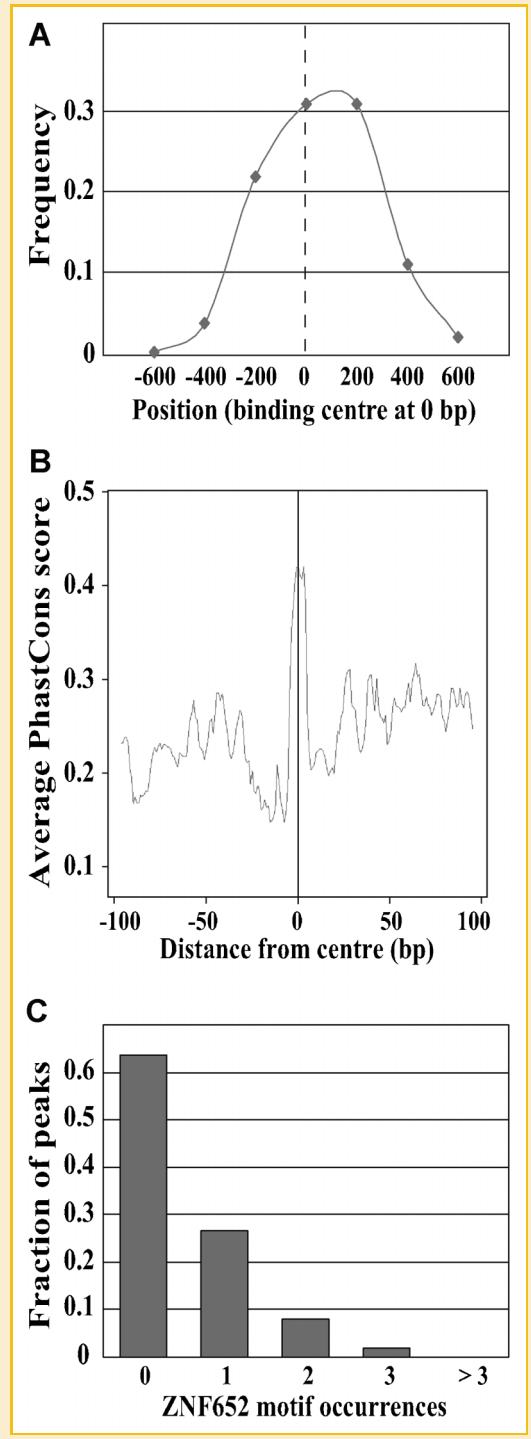

Fig. 4. A: Distribution of the ZNF652 consensus motif within binding sites (centre set at 0). B: Conservation plot of the ZNF652 promoter cistrome in vertebrate species (centre set at 0). Average PhastCons scores are based on alignment of binding sites with homologous genomic regions from chimpanzee, mouse, rat, dog, chicken, fungi and zebrafish. This analysis was performed using the Cistrome Analysis Pipeline (http://cistrome.dfci.harvard.edu/ap). C: Histogram representing the number of ZNF652 consensus motifs within all ZNF652 binding sites.

TABLE II. Enrichment of Known Motifs From the JASPAR Database [Bryne et al., 2008] in ZNF652 Binding Sites

ID	Name	Enrichment	*P*-value
All binding sites			
De novo ZNF652 motif	ZNF652	4.26	3.60E−16
MA0125.1	Nobox	2.17	8.56E−05
MA0040.1	Foxq1	2.21	3.48E−04
MA0060.1	NFYA	1.79	4.46E−04
MA0032.1	FOXC1	1.51	3.83E−03
MA0029.1	Evi1	3.37	5.13E−03
MA0137.2	STAT1	1.65	7.80E−03
MA0158.1	HOXA5	1.52	1.42E−02
MA0031.1	FOXD1	1.68	1.93E−02
MA0091.1	TAL1::TCF3	1.78	2.21E−02
MA0142.1	Pou5f1	1.78	2.86E−02
MA0007.1	AR	1.63	3.05E−02
MA0135.1	Lhx3	1.86	3.09E−02
MA0030.1	FOXF2	1.85	3.19E−02
MA0107.1	RELA	1.64	4.14E−02
MA0083.1	SRF	2.81	4.52E−02
Binding sites lacking de novo ZNF652 motif			
MA0112.2	ESR1	1.50	3.20E−04
MA0107.1	RELA	2.49	1.16E−03
MA0029.1	Evi1	4.26	4.68E−03
MA0018.2	CREB1	1.61	5.88E−03
MA0060.1	NFYA	1.77	6.44E−03
MA0091.1	TAL1::TCF3	2.10	1.12E−02
MA0061.1	NF-kappaB	1.75	1.52E−02
MA0007.1	AR	1.91	2.18E−02
MA0158.1	HOXA5	1.62	2.26E−02
MA0032.1	FOXC1	1.50	2.27E−02
MA0125.1	Nobox	1.78	3.19E−02
MA0109.1	Hltf	1.74	3.48E−02

syndromes and cancer [Vaquerizas et al., 2009]. Thus, functional characterisation of transcription factors is necessary to identify their downstream effectors, some of which may be exploitable as therapeutic targets. ZNF652 has been suggested to act in a transcriptional repressor complex with the ETO factor CBFA2T3 to repress transcription of oncogenic genes in the breast [Kumar et al., 2006]. In the present study, we report genome-wide mapping of ZNF652 promoter binding sites in a breast cancer cell line. This analysis has identified an in vivo ZNF652 recognition motif and revealed potential new direct gene targets of ZNF652, both of which are critical for understanding the function of this disease-associated transcription factor.

Identification of the sequences recognised by transcription factors, termed binding motifs, is a powerful means of identifying their target genes and thereby elucidating their function. However, only a small proportion of DNA motifs recognised by the many transcription factors encoded in the human genome have been characterised in vitro [Kummerfeld and Teichmann, 2006], and even fewer in vivo. In addition, nothing is known about the regulatory function of most of these transcription factors. The recent advances in ChIP-chip and ChIP-seq have enabled rapid and accurate identification of transcription factor binding motifs in vivo [Farnham, 2009]. This has provided significant insight into the functions of individual transcription factors and the mechanisms of transcriptional regulation as a whole [Farnham, 2009; Vaquerizas et al., 2009]. Here, we scanned the ZNF652 binding sites for de novo enriched sequences and identified a motif that closely matched the 10 bp core of the sequence that was previously reported to be recognised by ZNF652 in vitro. This 10 bp motif was found in approximately 36% of the ZNF652 binding sites. Interestingly, sites lacking the ZNF652 recognition motif showed similar levels of enrichment in the ChIP-chip assay (Fig. S1). There are at least three mechanisms by which ZNF652 could interact with promoters lacking this motif [Farnham, 2009]. First, it may be able to bind to another DNA sequence that was not detected using the de novo motif scanning methodology employed in this study. Recognition of different sequence motifs appears to be a common attribute of mammalian DNA binding proteins [Badis et al., 2009]. Characterisation of the full ZNF652 cistrome, rather than the promoter-specific cistrome reported here, may yield other ZNF652 DNA recognition sequences. Second, ZNF652 may interact with a distal site containing a consensus binding motif and loop to the site lacking a motif. Again, this mechanism could be revealed by characterisation of the full ZNF652 cistrome. Finally, ZNF652 may be tethered to sites lacking its recognition motif by other DNA binding proteins. Candidates for such a function include CREB1, NF-kappaB and the steroid hormone receptors ER and AR, the binding sites for which were shown to be differentially enriched in promoter sequences lacking a ZNF652 binding motif. Supporting the idea that ZNF652 and AR have a collaborative function in transcription, we have shown previously that a combination of high levels of both factors is associated with a statistically increased risk of prostate cancer relapse [Callen et al., 2010]. This information, combined with recent reports implicating AR in breast cancer [Tiefenbacher and Daxenbichler, 2008; Dimitrakakis and Bondy, 2009], indicates that further investigation into the potential interaction of ZNF652 and AR is warranted.

Many of the 113 genes with a ZNF652 binding site within their promoter have been implicated in different cancers. For example, breast (*ALDH18A1*, *ADCY6*, *RPIP8*, *H2AFX*, *PPP1R10*, *SPDEF*, *C6orf120* and *TRPS1*), prostate (*DUSP10*, *ALDH18A1*, *LDLR* and *TRPS1*), skin (*GRHL3*), hepatocellular carcinoma (*CENPE*) and non-Hodgkin's lymphomas (*H2AFX*). As limited data are available for the other genes with a ZNF652 binding site additional functional investigations will be required to establish their role in breast and other cancers. In addition, genome-wide mapping of the de novo motif revealed many more genes that are potentially regulated by ZNF652. The enrichment of signalling pathways that are involved in cancer development and progression within this gene set supports the idea that these genes may be targeted by ZNF652 in breast cancer. Validation of these targets could be achieved by future cistromic studies or by analysis of specific candidate genes.x

As a first step in examining the significance of specific ZNF652 cis-regulatory elements in breast cancer, we examined whether it binds differentially to three promoters (*PUS10*, *GRHL3* and *BGLAP*) in ZR75-1 cells compared to a normal mammary epithelial cell line, MCF10A. Interestingly, ZNF652 showed significantly higher enrichment at *GRHL3* in MCF10A cells. *GRHL3* encodes a pro-tumourigenic transcription factor of the mammalian grainyhead-like family of proteins [Guardiola-Serrano et al., 2008]. One possible explanation for this differential binding is that ZNF652 represses *GRHL3* in normal breast epithelial cells but this repression is lost or inhibited in cancer. We believe that more extensive comparisons of the malignant versus non-malignant ZNF652 cistromes will lead to the identification of novel oncogenes targeted by this factor.

The significance of in vivo fine mapping of transcription factor binding sites and its impact on understanding the regulatory networks and gene expression underlying growth and development is well recognised. Characterisation of the promoter-specific ZNF652 cistrome has uncovered a novel DNA motif recognised by this transcription factor and revealed potential genes under its direct transcriptional control. We envisage that this resource will provide a platform to further elucidate the function of ZNF652 in breast cancer. Moreover, these data can be used as a reference set for future work aimed at mapping ZNF652 DNA binding sites in other cell lines and/or tissues. Finally, these results will also facilitate investigations into the mechanism by which ZNF652 functions as a transcriptional repressor in the normal cell and how disruption of this function can lead to disease and cancer.

ACKNOWLEDGMENTS

We thank the National Breast Cancer Foundation, Australia for supporting research in our laboratory. Luke Selth is supported by a Young Investigator grant from the Prostate Cancer Foundation of Australia and a Freemason's Foundation Centre for Mens Health research fellowship. We also thank Shiwani Sharma for helpful discussions and critical reading of the manuscript.

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