Functional analysis of CBFA2T3: a breast cancer tumour suppressor from chromosome band 16q24.3

by

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To my lovely Son, Daughter

And my beloved Husband

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Abstract

Loss of heterozygosity (LOH) of 16q is an early event occurring in 36-60% of primary sporadic breast cancers. *CBFA2T3* (*MTG16*) is a putative breast cancer tumour suppressor gene, localized at chromosome band 16q24.3. CBFA2T3 (MTG16) belongs to the CBFA2T protein family and shares a high homology with other two members, CBFA2T1 (MTG8) and CBFA2T2 (MTGR1). CBFA2T1 and CBFA2T3 proteins form transcriptional repressor complexes with the DNA binding zinc finger proteins like BCL6, PLZF, Gfi1 and ZNF652. CBFA2T3 protein exists as isoform “a” and “b” that arise from alternate start sites. These isoform differ in their N-terminal sequences. Previous studies determined that CBFA2T3a localized to the nucleolus, while CBFA2T3b has a putative role as tumour suppressor protein.

The present study confirms that the database entries of CBFA2T3a are incomplete and an extended N-terminus region is present to CBFA2T3a (NCBI NM_005187) isoform by RT-PCR and DNA sequencing. Two rabbit polyclonal anti CBFA2T3 antibodies were raised against the region unique to CBFA2T3. These antibodies specifically detect the endogenous CBFA2T3 proteins and not CBFA2T1 and CBFA2T2. Cell fractionation studies show that endogenous CBFA2T3a localized to the cytoplasm, while CBFA2T3b targeted to the nucleus. The N-terminus region specific to “a” isoform determined the cytoplasmic localization. The detailed studies show that CBFA2T3a localized to centrosome and this was confirmed by co–localization with known centrosomal proteins γ-tubulin. This was further confirmed by immunoprecipitation of γ-tubulin with N-terminus regions of CBFA2T3a protein. Further investigation showed that CBFA2T3a localizes to

the centrosome through out the centrosomal duplication. Presence of CBFA2T3a on procentriole was further confirmed by co-localization with known proteins having a crucial role in centrosome duplication like HsSAS6 and polyglutamilated tubulin.

Experiments were conducted to determined if the different subcellular localization of “a” and “b” isoforms resulted into functional differences between two isoforms. Immunoprecipitation experiments with known DNA binding proteins like BCL6 and PLZF showed that CBFA2T3b interacts with BCL6, while no interaction was found with PLZF. Consistent with the known transcriptional co-repressor function, real time RT-PCR showed that CBFA2T3b has an additive effect on BCL6 mediated repression of its target cyclin D2, while no effect was observed with CBFA2T3a. Real time RT-PCR data also showed that BCL6 not only recruits CBFA2T3b to repress its target but also have repressive effects on CBFA2T3 transcription. CBFA2T3b transcription regulation by BCL6 was found to be mediated through one or two BCL6 putative binding sites in CBFA2T3b promoter.

Immuno histochemical studies were carried out to analyse CBFA2T3b function as a breast cancer tumour suppressor. CBFA2T3 proteins are highly expressed in epithelial cell lineage of normal breast ducts, while its expression is lost in some tumours. CBFA2T3 expression was further analysed in a cohort of commercially available breast tumour sections. Data from these studies showed the loss of CBFA2T3 nuclear expression in some tumours, which was significantly correlated with tumours positive for HER2 expression, molecular subtypes and histological staging of the tumours. CBFA2T3 cytoplasmic expression was also down regulated in tumour sections. A significant association of CBFA2T3 cytoplasmic expression was observed with the TNM grading for tumour invasion and centrosomal abnormalities in BR701 TMA.

Knock down studies using shRNA were conducted to investigate the role of CBFA2T3a. Following CBFA2T3 knock down in cells with minimal CBFA2T3b expression, an increase in centrosomal abnormalities was observed. These abnormalities were associated with a significant increase in metaphase anomalies. Since the “a” isoform is localized to cytoplasm and particularly centrosome, it was considered that this isoform is determining centrosome integrity.

This work has provided a new insight into the localization pattern of CBFA2T3 isoforms, as CBFA2T3a and b isoforms were localized to different cellular compartments and were involved in distinct functions. CBFA2T3b function as a transcriptional co repressor, CBFA2T3b expression was lost in a group of breast tumours sections. Given that CBFA2T3a has a critical centrosomal function, the expression of this isoform would be expected to be maintained, even in the absence of the CBFA2T3b isoform in tumours. CBFA2T3a specific knock down studies may give a full insight on direct targets of CBFA2T3a, having a controlling role in normal centrosome duplication cycle.

Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abbreviations

AD – Activator domain

ADH – Atypical ductal hyperplasia

AML – Acute myeloid leukaemia

BAC – Bacterial artificial chromosome

B6BS – BCL6 binding sites

CBFA2T3 – Core-binding factor, runt domain α subunit 2; translocated to 3

CBFA2T1 – Core-binding factor, runt domain α subunit 2; translocated to 1

cDNA – complementary DNA

ChIP – Chromatin Immunoprecipitation

DAPI - 4',6-diamidino-2-phenylindole

DCIS – Ductal carcinoma *in situ*

DNA – deoxyribonucleic acid

DSB – double-strand breaks

DTT - Ditheothiol

E2 - Estradiol

ER – Estrogen receptor

ERE – Estrogen response element

EGF – epidermal growth factor

EGFP – enhanced green fluorescent protein

EGFR – Epidermal growth factor receptors

FCS – Fetal calf serum

GFP – Green fluorescent protein

HA - hemagglutinin

HDAC – Histone deacetyltransferase

HMEC – Human mammary epithelial cell

IDC – Invasive ductal carcinoma

IF – Immunofluorescence

IHC - Immunohistochemistry

ILC – Invasive lobular carcinoma

IP - Immunoprecipitation

LCIS – Lobular carcinoma *in situ*

LOH – Loss of heterozygozity

mRNA – messenger RNA

MTG16 – Myeloid transforming gene from chromosome 16 protein

MTG8 – Myeloid transforming gene from chromosome 8

MTGR – Myeloid transforming gene related protein-1

NCBI - National Center for Biotechnology Information

NES – Nuclear export sequences

NR – Nuclear receptor

NLS – Nuclear localisation signal

ONC – Oncogene

PCR – Polymerase chain reaction

PEST sequence – Proline, glutamic acid, serine and threonine rich sequence

PR – Progesterone receptor

Real-time RT-PCR – Reverse transcription real time-PCR

RD – Repressor domain

RNA – ribonucleic acid

SDS – Sodium dodecylsulphate

SDS-PAGE – SDS Polyacrylamide gel electrophoresis

SEM – Standard error of the mean

shRNA – Short hairpin RNA

siRNA – Small interfering RNA

SNP – Single nucleotide polymorphism

SRO – Smallest region of overlap

TSG – Tumour suppressor gene

WB – western blot

Y2H – yeast-2-hybrid

α-FLAG - anti-FLAG

α-Myc – Anti-myc antibody

Publications

In preparation:

1 **Saif Z**, Millband D, Ricciardelli C, Kumar R and Callen DF (2009). CBFA2T3a isoform is functionally distinct from CBFA2T3b (Manuscript in internal review)

2 **Saif Z**, Millband D, Ricciardelli C, Kumar R and Callen DF (2009). Evaluating CBFA2T3 as a novel prognostic marker for breast cancer. (Manuscript in preparation)

Conference presentations:

1 **Z Saif** , D Millband, C Ricciardelli, R Kumar and D F Callen. A discrete role for CBFA2T3a isoform in centrosome function, in contrast to CBFA2T3b: a breast tumour suppressor protein. July 22, 2008 Post graduate research Expo, Faculty of Health Sciences, The Adelaide University, Adelaide.

2 **Z Saif** , D Millband, C Ricciardelli, R Kumar and D F Callen. Evaluating CBFA2T3, a putative tumour suppressor from chromosome band 16q24.3, as a novel prognostic marker for breast cancer. April1-4 2006, 97th annual AACR meeting, Washington DC, USA.

3 **Z Saif**, J Forsyth, D Millband, P M Neilsen, J Lee, R Kumar and D F Callen. Functional studies of CBFA2T3, a putative breast tumour suppressor from chromosome band 16q24.3. Feb 2005, Lorn cancer meeting, Philip island,