Original article

Association of mutation and low expression of the CTCF gene with breast cancer progression

Md. Salman Akhtar a,b, Naseem Akther a,b, Mohammad Zeeshan Najm c, S.V.S Deo d, N.K. Shukla d, Shaia Saleh R. Almalkib, Raed A. Alharbib, Abdulmajeed Abdulghani A. Sindi b, Abdulmohsen Alruweteie, Abrar Ahm df, Syed Akhtar Husaina,⇑

a Human Genetics Laboratory, Department of Biosciences, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India
b Faculty of Applied Medical Sciences, Albaaha University, Albaaha, Saudi Arabia
c Department of Biochemistry, Jamia Hamdard, New Delhi 110062, India
d Department of Surgical Oncology, DR. BRA-IRCH, AIIMS, New Delhi 110029, India
e Department of Medical Laboratory, College of Applied Medical Sciences, Qassim University, Qassim, Saudi Arabia
f Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

A R T I C L E   I N F O

Article history:
Received 10 December 2019
Accepted 29 March 2020
Available online 2 April 2020

Keywords:
Breast cancer
CTCF
Immunohistochemistry
Mutation
PCR-SSCP

A B S T R A C T

Background: CTCF encodes 11-zinc finger protein which is implicated in multiple tumors including the carcinoma of the breast. The Present study investigates the association of CTCF mutations and their expression in breast cancer cases.

Methods: A total of 155 breast cancer and an equal number of adjacent normal tissue samples from 155 breast cancer patients were examined for CTCF mutation(s) by PCR-SSCP and automated DNA sequencing. Immunohistochemistry (IHC) method was used to analyze CTCF expression. Molecular findings were statistically analyzed with various clinicopathological features to identify associations of clinical relevance.

Results: Of the total, 16.1% (25/155) cases exhibited mutation in the CTCF gene. Missense mutations Gln > His (G > T) in exon 1 and silent mutations Ser > Ser (C > T) in exon 4 of CTCF gene were analyzed. A significant association was observed between CTCF mutations and some clinicopathological parameters namely menopausal status (p = 0.02) tumor stage (p = 0.03) nodal status (p = 0.03) and ER expression (p = 0.04). Protein expression analysis showed 42.58% samples having low or no expression (+), 38.0% with moderate (++) expression and 19.35% having high (+++) expression for CTCF. A significant association was found between CTCF protein expression and clinicopathological parameters include histological grade (p = 0.04), tumor stage (p = 0.04), nodal status (p = 0.03) and ER status (p = 0.04).

Conclusions: The data suggest that CTCF mutations leading to its inactivation significantly contribute to the progression of breast cancer.

© 2020 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Breast cancer, that represents nearly 1/4th of total cancers diagnosed in female (Ferlay et al., 2015), results from the interaction between multiple genes and environmental factors (Kaaks et al., 2005) (Xie et al., 2006).

CTCF (CCCTC-binding factor) regulates gene expression through activation/repression of the promoter, chromatin lining and imprinting of genomes (Ong and Corces, 2014), along with involvement in the establishment of the 3D structure of the genome and genomic segments (Holwerda and de Laat, 2013). It also has a tumor suppressor function and is commonly deleted or mutated in breast cancer cell lines and breast tumors (Ji et al., 2016; Kaiser et al., 2016; Sabarinathan et al., 2016; Umer et al., 2016).

11-zinc fingers enable the protein to bind diverse gene sequences making it a universal transcription factor (Kim et al., 2007; Nakahashi et al., 2013). CTCF is a tumor suppressor suspects linked with familial breast cancer (Ohlsson et al., 2001). Genomic
platforms and integration of sequence data suggest that CTCF may harbor driver mutation in breast cancer (Cancer Genome Atlas, 2012; Nik-Zainal et al., 2016). CTCF protein levels are observed to be upregulated in many breast tumors as well as cancer cell lines (Docquier et al., 2005). The epigenetic control of the BAX gene by CTCF helps the cancer cells to evade apoptosis in addition to influencing genome imprinting, intronic transcription, inactivation of X-chromosome, and post-transcription processing (Docquier et al., 2005; Mendez-Catala et al., 2013). It further downregulates the transcription of the c-Myc oncogene and impacts the expression of maternal H19 allele (Holmgren et al., 2001), as well as establishes chromatin boundaries and mediating long-range chromatin interactions (Phillips and Corces, 2009).

16q22-24 region, the location of CTCF, commonly exhibits loss of heterozygosity (LOH) in breast cancer (Lindblom et al., 1993, Clifton-Jansen et al., 1994). Interestingly, this phenomenon is associated with longer survival and delayed metastasis (Lindblom et al., 1993; Hansen et al., 1998).

CTCF can be deregulated in multiple ways, including germline and somatic (missense and nonsense) mutations resulting in the onset and progression of genetic disorders like human cancers (Lupianez et al., 2015; Filippova et al., 1998; Rubio-Perez et al., 2015). The CTCF gene mutation(s) leading to many human cancers strongly suggest the critical loss of function as important as tumor suppression (Marshall et al., 2017; Ohlsson et al., 2001; Filippova et al., 1998).

An early in vitro report indicated the anti-proliferative activity of CTCF by showing that it repressed the cell proliferation (Lutz et al., 2000). However, the precise role of CTCF in the onset or progression of cancer remains to be elucidated. The efforts have been made in many human tumors, but breast cancer is scarcely studied (Takai et al., 2001, Filippova et al., 2002, Yeh et al., 2002, Aulmann et al., 2003, Ulaner et al., 2003).

The current study aimed to find mutations in various hot spot exons of CTCF by polymerase chain reaction-single stranded conformation polymorphism (PCR-SSCP) followed by sequencing of DNA isolated from cancerous and adjacent normal breast tissues to identify mutational hot spots of CTCF contributing to carcinogenesis. Additionally, the expression of CTCF protein was also analyzed to show the relationship between CTCF mutation and its expression with various clinico-pathological parameters.

2. Materials and Methods

2.1. Biological samples

The subjects were informed, and consent forms were collected from all the participants after the ethical clearance from the institutional ethics committee of All India Institute of Medical Sciences (AIIMS), New Delhi, India. 155 female breast cancer tissue samples and an equal number of adjacent normal tissues (measuring 5–10 mm), were obtained and stored in PBS and formalin between 2010 and 2014 from the Department of Surgical Oncology, AIIMS, New Delhi India. Breast cancer stages were determined using the TNM staging system or American Joint Committee on Cancer (AJCC). The clinico-pathological variables are age, histological type, tumor size, histological grade, menopausal status, tumor stage, nodal status, ER expression, PR expression and Her2/Neu.

2.2. DNA isolation

DNA was isolated from the cases and healthy control tissue by using the standard phenol/chloroform method as described previously (Sambrook et al., 1989). DNA concentration and purity were determined using electrophoresis and UV spectrophotometry before storing it in TE buffer.

2.3. PCR-SSCP analysis

Hot spot exon 1 and 4 of CTCF gene were probed for the presence of mutation using the tumor and normal control DNA with the primers (table 1). PCR product showed the presence of 206 bp and 246 bp amplicons [Fig. 1] as identified by using QuantOne Software (Bio-Rad Laboratories, CA, USA). The purified product was assayed for any alteration in the electrophoretic mobility as described previously (Orita et al., 1989). Comparison of single-stranded DNA bands of the tumor and normal control to the identification of SSCP positive samples (Fig. 2).

2.4. DNA sequencing

The SSCP positive samples were re-amplified and purified before sequencing twice to prevent the formation of any artifacts (ABI PRISM310 dye Terminator Cycle Sequencing Ready reaction Kit) and analyzed using Sequencing Analysis Software 3.4.1 (Fig. 3).

2.5. IHC analysis

Immunohistochemical staining was done to assess CTCF protein expression using the anti-human CTCF antibody (GeneScript, USA, Catalog # A01529) (Barbarestchi et al., 1996). Briefly, breast cancer tissue samples cut into 2 to 4 μm section embedded in poly-L-lysine coated slides were treated with xylene, alcohol, and heat to retrieve the antigen. The slides were finally incubated with anti-CTCF antibody and developed using streptavidin Horse-redish peroxidase detection kit (GeneScript USA). Slides scoring as Low or no expression (+), Moderate (++) and High expression (+++) representation of number and distribution of cells among cases and controls was done using Olympus BX 50, Tokyo.

2.6. Statistical analysis

Chi-square test ($\chi^2$) was used to assess the association of CTCF mutation and its expression with various clinico-pathological parameters using GraphPad Prism 6.0. The P-value $\leq 0.05$ was considered significant.

3. Results

3.1. Mutation(s) in CTCF and clinico-pathological parameters

A total of 25 (16.12%) breast cancer cases showed mutations in the exon 1 and exon 4 including a missense mutation (Gln > His, G > T) in seventeen cases and silent mutation (Ser > Ser, C > T) in eight cases (Table 2) (Fig. 6).

| Table 1 | Oligonucleotide primer sequences used for amplification of different exons. |
|---------|--------------------------------------------------|
| Gene    | Consensus sequence                  | Annealing temperature ($^\circ$C) | Amplicon size (bp) |
| CTCF Exon 1 FP | 5’-GGTGATGATGGAACAGCTTG-3’ | 60 | 206 |
| CTCF Exon 1 RP | 5’-TGGTAGCAACAGTACAGTC-3’ | 58 | 246 |
| CTCF Exon 4 FP | 5’-CCGAGACGATTCCTAATCACC-3’ | 58 | 246 |
| CTCF Exon 4 FP | 5’-TACATTGCTTCCTCAGTCCGG-3’ | 58 | 246 |
No mutation was observed in normal control samples. The relationship between CTCF mutations and clinicopathological parameters showed a significant correlation with patients menopausal status ($p = 0.02$), tumor stage ($p = 0.03$), nodal status ($p = 0.03$) and estrogen receptor (ER) expression ($p = 0.04$ (Table 3). However, the association with age, histological type, tumor size, histological grade, progesterone receptor (PR) expression, and Her2/Neu failed to reach statistical significance.

3.2. CTCF expression and clinicopathological features

Of all 155 cases, 66 (42.59%) showed low/no expression (+), 59 cases (38.06%) with moderate (++) expression and 30 cases (19.35%) had high (++++) expression for CTCF nuclear staining (Table 4, Fig. 5). A significant correlation was detected between CTCF protein expression and histological grade ($p = 0.04$), nodal status ($p = 0.03$), tumor stage ($p = 0.04$), and ER status ($p = 0.04$) (Table 5). However, the association with age ($p = 0.29$), menopausal status ($p = 0.84$), histological type ($p = 0.56$), tumor size ($p = 0.464$), PR status ($p = 0.10$) and Her2/Neu ($p = 0.49$) failed to reach significance (Table 5).

3.3. Correlation between mutation(s) and expression of CTCF cases

The mutation(s) found in the breast cancer patients was analyzed along with CTCF expression to elucidate the potential role of CTCF in breast cancer. The relationship of CTCF mutation with its expression was observed significantly in the case of low level (+) protein expression ($p = 0.03$). However, the link in cases of moderate (++) ($p = 0.11$) and high level (++++) ($p = 0.43$) protein expression was found not significant (Table 6).
4. Discussion

Aberrant CTCF is linked with several diseases/disorders, including cancer (Aulmann et al., 2003; Prawitt et al., 2005; Herold et al., 2012; Gregor et al., 2013; Bastaki et al., 2017). The tumor suppressor function of CTCF is speculated based upon its impact on critical genes like p53, Myc, BRCA1, p19/ARF involved in cancer onset and progression (Bell and Felsenfeld, 2000, Klenova et al., 2002, Qi et al., 2003, Ohlsson et al., 2001).

Recent studies suggest that overexpression of CTCF contributes to tumor development in breast cancer by downregulating HOXA10 and H3K27me3 expressions (Mustafa et al., 2015; Lee et al., 2017). Interestingly, the repression of CTCF leads to the overexpression of BAX and eventual apoptosis (Docquier et al., 2005).

Table 2
Details of CTCF gene Mutation(s) in Female Breast Cancer Cases from India.

| Affected Codon | Base Position | Base Change | Amino Acid Change | Mutation Effect | No. of Patients |
|---------------|--------------|-------------|-------------------|----------------|----------------|
| 72            | 216          | CAG > CAT (G > T) | Glutamine > Histidine (Gln > His) | Missense | 17             |
| 388           | 1455         | TCC > TCT (C > T) | Serine > Serine (Ser > Ser) | Silent   | 08             |

Table 3
Correlation between mutations of human CTCF gene with clinicopathological parameters.

| Parameters                              | No. of cases (n = 155) | Mutations | Mutation rate (%) | \(\chi^2\) value | P value |
|-----------------------------------------|------------------------|-----------|-------------------|-----------------|---------|
| Age                                     |                        |           |                   |                 |         |
| >50                                     | 80                     | 16        | 20.00             | 1.831           | 0.176   |
| ≤50                                     | 75                     | 09        | 12.00             |                 |         |
| Menopausal status                       |                        |           |                   |                 |         |
| Pre                                     | 70                     | 06        | 8.57              | 5.390           | 0.020** |
| Post                                    | 85                     | 19        | 22.35             |                 |         |
| Histological Type                       |                        |           |                   |                 |         |
| Invasive ductal Carcinoma (IDC)         | 150                    | 25        | 16.67             | 0.993           | 0.318   |
| Invasive lobular carcinoma (ILC)        | 00                     | 00        | 05                |                 |         |
| Tumor Size                              |                        |           |                   |                 |         |
| ≤2cm                                    | 65                     | 07        | 10.77             | 2.377           | 0.123   |
| >2cm                                    | 90                     | 18        | 20.00             |                 |         |
| Histological Grade                      |                        |           |                   |                 |         |
| Poorly differentiated (PD)              | 40                     | 05        | 12.50             | 1.596           | 0.450   |
| Moderately differentiated (MD)          | 69                     | 14        | 20.29             |                 |         |
| Well differentiated (WD)                | 46                     | 06        | 13.04             |                 |         |
| Tumor Stage                             |                        |           |                   |                 |         |
| Stage II (a+b)                          | 73                     | 07        | 09.59             | 4.363           | 0.036** |
| Stage III (a+b) + IV                    | 82                     | 18        | 21.95             |                 |         |
| Nodal Status                            |                        |           |                   |                 |         |
| Positive                                | 81                     | 18        | 22.22             | 4.656           | 0.030** |
| Negative                                | 74                     | 07        | 09.46             |                 |         |
| Estrogen Receptor (ER) Expression       |                        |           |                   |                 |         |
| Positive                                | 72                     | 07        | 09.72             | 4.080           | 0.043** |
| Negative                                | 83                     | 18        | 21.68             |                 |         |
| Progesterone Receptor (PR) Status       |                        |           |                   |                 |         |
| Positive                                | 66                     | 08        | 12.12             | 1.365           | 0.242   |
| Negative                                | 89                     | 17        | 19.10             |                 |         |
| Her2/Neu                                |                        |           |                   |                 |         |
| Positive                                | 69                     | 07        | 10.14             | 3.292           | 0.069   |
| Negative                                | 86                     | 18        | 20.93             |                 |         |

P-value < 0.05** was considered significant.

Table 4
Profile of CTCF protein expression.

| CTCF gene expression | Low 66/155 | Moderate 59/155 | High 30/155 |
|----------------------|-----------|----------------|-----------|
|                      | 42.59%    | 38.06%         | 19.35%    |

Mutations have been detected in CTCF chromatin binding sites (CBS) in multiple cancers, especially mutations of A-T base pairs (Katainen et al., 2015). The loss of CTCF poly ADP-riboseylation in breast cancer leading to the expression of both 180-kDa and 130-kDa in comparison to only 180-kDa CTCF in normal breast tissues likely contributes to the progression of breast cancer (Docquier et al., 2009).

Although CTCF-130-kDa or Rb2/p130 can be used as the biomarker for the cancer progression, the utility of CTCF-130-kDa as the disease prognosis biomarkers remain to be investigated (Long et al., 2018; Shi et al., 2018; Wang et al., 2017; Wu et al., 2018; Kawamura et al., 2018). Recent studies show that CTCF regulates changes of the 3D genome organization (Wang, 2018; Singh and Shrivastava, 2017, Liu and Hanada, 2018).

In the present study, we screened the hotspot coding regions of CTCF gene for the mutation(s) by PCR-SSCP in 155 cases of female breast carcinoma along with corresponding adjacent healthy control. We found 25 (16.1%) missense and silent mutations in female breast cancer tissues as shown in the table 2. The mutation(s) were identified at codon 72 leading to Gln > His (G > T), and at codon 1455 leading to Ser > Ser(C > T). The missense codon mutations
in **CTCF** zinc finger domain 3, observed in the present study may impact the CTCF binding to the promoters of genes related to cellular proliferation like **MYC**, **PLK**, **PIM-1**, **p19ARF**, and **Igf2/H19** (Fig. 4) (Filippova et al., 2002). The mutation(s) may also have resulted in repression of the wild-type allele and low/no protein expression (Aulmann et al., 2003). Moreover, our data exhibited
the altered expression profiles of CTCF which may be due to the result of potential mutation(s) in the CTCF exonic region and thus can contribute in the progression of breast cancer as shown in an early study (Tiffen et al., 2013).

Importantly, we observed that CTCF mutations were only found in breast cancer tissue and not in healthy tissues. The analysis of potential relationship with the patient’s ages, menopausal status, histological types, tumor sizes, histological grades, tumor stages, lymph node metastases, steroid receptors (ER & PR) and Her2/neu amplifications to elucidate the role of mutation(s) in CTCF gene in the progression of breast cancer revealed a significant relationship between CTCF mutations and patients’ menopausal status (p = 0.02), tumor stages (p = 0.03), lymph node metastases (p = 0.03) and ER (p = 0.04). The significant association with the clinical parameters further emphasizes the link between CTCF and breast cancer progression. No significant association was found with parameters such as age, menopausal status, histological types, tumor sizes, PR status and Her2/neu amplifications of breast cancer progression (Table 5) suggesting the involvement of detected mutations in expression and altered formations of the protein contributing to the onset and progression of breast cancer which is in agreement with the findings of an early study (Tiffen et al., 2013).

A significant association found between CTCF mutation and protein expression in the low level (+) category of protein expression (p = 0.03), whereas no significant association with the moderate (+) and high level (+++) of protein expression suggest that the progression of breast cancer may be related to the lower level of CTCF protein expression indicating towards the anti-proliferative activity of CTCF, and a potent breast cancer susceptible genes. The altered expression profiles of CTCF showing mainly nuclear expression in the current study are in agreement with the earlier studies using immunofluorescence to without any cytoplasmic staining (Zhang et al., 2004). In the present study, we found an association between CTCF expression and histological grade in the high percentage of low-grade tumors having less proliferative activity showing positive nuclear expression, whereas high-grade tumors primarily showed low or no expression. Our results are concordant with early reports indicating the ability of CTCF to inhibit cell growth and proliferation (Rasko et al., 2001).

Table 5
Correlation between the expression of CTCF protein and Clinico-pathological parameters of breast carcinoma.

| Parameters               | n=155 | Low | Normal | High | $\chi^2$ | p value |
|-------------------------|-------|-----|--------|------|----------|---------|
| Age                     |       |     |        |      |          |         |
| >50                     | 80    | 35  | 14     | 31   | 2.447    | 0.294   |
| ≤50                     | 75    | 29  | 21     | 25   |          |         |
| Menopausal status       |       |     |        |      |          |         |
| Pre                     | 70    | 32  | 14     | 24   | 0.328    | 0.848   |
| Post                    | 85    | 35  | 18     | 32   |          |         |
| Histological Type       |       |     |        |      |          |         |
| Invasive Ductal Carcinoma (IDC) | 150   | 60  | 39     | 51   | 1.158    | 0.560   |
| Invasive Lobular Carcinoma (ILC) | 05    | 02  | 02     | 01   |          |         |
| Tumour Size             |       |     |        |      |          |         |
| ≤2cm                    | 65    | 23  | 18     | 24   | 1.532    | 0.464   |
| >2cm                    | 90    | 40  | 24     | 26   |          |         |
| Histological Grade      |       |     |        |      |          |         |
| First                   | 40    | 20  | 09     | 11   | 09.791   | 0.044** |
| Second                  | 69    | 23  | 25     | 21   |          |         |
| hird                    | 46    | 12  | 11     | 23   |          |         |
| Tumor stage             |       |     |        |      |          |         |
| Stage2 (a+b)            | 73    | 23  | 18     | 32   | 6.079    | 0.047** |
| Stage3 (a+b) +4         | 82    | 38  | 23     | 21   |          |         |
| Nodal status            |       |     |        |      |          |         |
| Positive                | 81    | 38  | 21     | 22   | 6.824    | 0.033** |
| Negative                | 74    | 24  | 15     | 35   |          |         |
| ER status               |       |     |        |      |          |         |
| Positive                | 72    | 22  | 17     | 33   | 6.008    | 0.049** |
| Negative                | 83    | 40  | 19     | 24   |          |         |
| PR Status               |       |     |        |      |          |         |
| Positive (*+ve)          | 66    | 36  | 12     | 18   | 4.475    | 0.106   |
| Negative (−ve)          | 89    | 34  | 26     | 29   |          |         |
| Her2/Neu                |       |     |        |      |          |         |
| Positive (*+ve)          | 69    | 24  | 19     | 26   | 1.413    | 0.493   |
| Negative (−ve)          | 86    | 38  | 20     | 28   |          |         |
5. Conclusions

Our study showed mutations (missense Gln > His, G > T and silent Ser > Ser, C > T) of the CTCF gene in Indian female breast cancer cases. The detected mutations showed that 16 (64%) mutations had a statistically significant association with low or no expression for CTCF when analyzed with IHC data. The findings suggest that CTCF may be a tumor suppressor gene and its inactivation may play an essential role in the progression of breast carcinoma. However, further clinical studies with larger sample size are needed to elucidate the fundamental role of CTCF gene in the breast cancer onset and progression in Indian population.

Acknowledgments

The author would like to thank the Indian Council of Medical Research (ICMR), Government of India, New Delhi India (grant number 3/2/261/2011/MCD3), for providing the fund for this study. The authors would like to thank all the breast cancer patients who participated in the study, and without whom the study would not have been possible.

Declaration of Competing Interest

All authors have read and approved the final manuscript and there is no conflict of interest.

References

Aulmann, S., Blaker, H., Penzel, R., Rieker, R.J., Otto, H.F., Sinn, H.P., 2004. Comprehensive molecular portraits of human cancer. Cancer Res. 64, 8982–9031.

Barbareschi, M., Caffo, O., Veronesi, S., Leek, R.D., Fina, P., Fox, S., Sonzani, M., Girlando, S., Morelli, L., Egger, C., Pezzella, F., Dogliotti, C., Dalla Palma, P., Harris, A., 1990. Bcl-2 and p53 expression in node-negative breast cancer patients. Cancer Res. 50, 5497–5502.

Bastaki, F., Nair, P., Mohamed, M., Malik, E.M., Helmi, M., Al-Al, M.T., Hamzeh, A.R., 2017. Identification of novel CTCF mutation responsible for syndromic intellectual disability - a case report. BMC Med. Genet. 18, 68.

Bell, A.C., Felsenfeld, G., 2000. Methylation of a CTCF-dependent boundary controls selective alteration of tSS DNA-binding specificity. Cancer Res., 62, 48–52.

Filippova, G.N., Qi, C.F., Ulmer, J.E., Moore, J.M., Ward, A.D., Uebe, S., Vasileiou, G., Reis, A., Zhou, H., Zweier, C., 2013. De novo mutations in the genome organizer CTCF cause intellectual disability. Am. J. Hum. Genet. 93, 124–131.

Hansen, L.L., Yilmaz, M., Overgaard, J., Andersen, J., Kruse, T.A., 1998. Allelic loss of 16q23.2-24.2 is an independent marker of good prognosis in primary breast cancer. Cancer Res. 58, 2166–2169.

Herold, M., Barilliu, M., Revaz, V., 2012. CTCF: insights into insulator function during development. Development, 139, 1045–1057.

Holmgren, C., Kanduri, D., Delli, G., Ward, A., Mukhopadhyay, R., Kanduri, M., Lobanenkov, V., Ohlsson, R., 2001. CpG methylation regulates the Igf2/H19 insulin. Mol. Cell. Biol. 11, 1129–1130.

Holwerda, S.J., de Laat, W., 2013. CTCF: the protein, the binding partners, the binding sites and their chromatin loops. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20120369.

Kates, R., Kral, K., Qin, D., Pu, Z., Ziegler, A., Funk, E., Fox, S., Bonzanini, M., A, S., Hnizd, D., Pogorelov, G., Lee, T.I., Misteli, T., Janisch, R., Young, R.A., 2016. 3D Chromosome Regulatory Landscape of Human Pluripotent Cells. Stem Cell. Cell 18, 267–275.

Kaja, R., Rinaldi, S., Key, T.J., Berrino, F., Peeters, P.H., Biesy, C., Dossus, L., Lukanova, A., Bingham, S., Khaw, K.T., Allen, N.E., Bueno-De-Mesquita, H.B., van Gilis, C.H., Grobbée, D., Boeijing, H., Lahmann, P.H., Nagel, G., Chang-Claude, J., Clavel-Chapelon, F., Fournier, A., Theuern, A., Gonzalez, C.A., Quirós, J.R., Tormo, M.J., Ardanza, E., Azziz, P., Krogh, V., Palmi, D., Panso, S., Turino, R., Vines, P., Trichopoulou, A., Kalapothaki, V., Trichopoulou, D., Ferrari, P., Norat, T., Saracci, R., Riboli, E., 2005. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. Endocr. Relat. Cancer, 12, 1231–1245.

Klenova, E.M., Morse, H.C., Ohlsson, R., Lobanenkov, V.V., 2002. The novel BORIS + CTCF gene family is uniquely involved in the epigenetics of normal biology and cancer. Semin. Cancer Biol., 12, 399–414.

Lee, J.Y., Mustafa, M., Kim, C.Y., Kim, M.H., 2017. Depletion of CTCF in Breast Cancer Cells Selectively Induces Cancer Cell Death via p53. Cancer 8, 2124–2131.

Lobanenkov, V., Aulmann, S., Kral, K., Qin, D., Pu, Z., Ziegler, A., Funk, E., Fox, S., Bonzanini, M., A, S., Hnizd, D., Pogorelov, G., Lee, T.I., Misteli, T., Janisch, R., Young, R.A., 2016. 3D Chromosome Regulatory Landscape of Human Pluripotent Cells. Stem Cell. Cell 18, 267–275.

Maartens, E.T., Ackermann, M., Van, P., Caffo, O., Veronesi, S., Leek, R.D., Fina, P., Fox, S., Sonzani, M., Girlando, S., Morelli, L., Egger, C., Pezzella, F., Dogliotti, C., Dalla Palma, P., Harris, A., 1990. Bcl-2 and p53 expression in node-negative breast cancer patients. Cancer Res. 50, 5497–5502.

Mandal, A., Alldridge, L., Klenova, E., 2009. Decreased poly(ADP-ribosyl)ation of CTCF in breast cancer patients. Cancer Res. 69, 1555–1566.

M.Q., Lobanenkov, V.V., Ren, B., 2007. Analysis of the vertebrate insulator gene in breast cancer. Cancer Res. 67, 1231–1239.

Nelakuditi, V., Kaelin, W.G., Jr., 2006. The tumor suppressor protein p53 and its role in breast cancer. Breast Cancer Res. Treat. 99, 3–14.

Paigen, K., 1987. On the role of p53 in breast cancer. Cancer Res. 47, 2535–2538.

Petterson, N., Sjöblom, T., De, H., Hirota, K., Parmigiani, G., Lin, A., Leach, D., Gabriel, S., Barlow, D., Weir, B., 2006. Somatic mutations in the CTCF gene in human cancers. Cancer Res. 66, 5472–5476.

Pollack, J.R., 1999. The role of p53 in breast cancer. Cancer Res. 59, 1879–1883.

Poulsen, M.H., 2013. De novo mutations in the genome organizer CTCF cause intellectual disability. Am. J. Hum. Genet. 93, 124–131.

Qin, D., Pu, Z., Ziegler, A., Funk, E., Fox, S., Bonzanini, M., A, S., Hnizd, D., Pogorelov, G., Lee, T.I., Misteli, T., Janisch, R., Young, R.A., 2016. 3D Chromosome Regulatory Landscape of Human Pluripotent Cells. Stem Cell. Cell 18, 267–275.

Pu, Z., Ziegler, A., Funk, E., Fox, S., Bonzanini, M., A, S., Hnizd, D., Pogorelov, G., Lee, T.I., Misteli, T., Janisch, R., Young, R.A., 2016. 3D Chromosome Regulatory Landscape of Human Pluripotent Cells. Stem Cell. Cell 18, 267–275.

Rütten, E., 2002. The role of p53 in breast cancer. Cancer Res. 59, 1879–1883.
Rasko, J.E., Klenova, E.M., Leon, J., Filippova, G.N., Loukinov, D.I., Vatolin, S., Qi, C.F., Martensson, A., Mattioli, M., Dalla-Favera, R., Lobanenkov, V.V., Morse, H.C., Phillips, J.E., Corces, V.G., 2009. CTCF: master weaver of the genome. Cell 137, 1194–1889.

Ohlsson, R., Renkawitz, R., Lobanenkov, V., 2001. CTCF functions as a critical regulator of cell-cycle arrest and death of somatic mutations in 560 breast cancer whole-genome sequences. Nature, 467, 265–267.

Singh, S., Shrivastava, A.K., 2017. In silico characterization and transcriptomic analysis of nif family genes from Anabaena sp. PCC7120. Cell Biol. Toxicol. 33, 163–166.

Wang, X. 2018. Clinical trans-omics: an integration of clinical phenomes with molecular multiomics. Cell Biol. Toxicol., 34, 423–427.

Wang, X. 2018. Clinical trans-omics: an integration of clinical phenomes with molecular multiomics. Cell Biol. Toxicol., 34, 423–427.

Wu, D., Wang, X., Sun, H. 2018. The role of mitochondria in cellular toxicity as a potential drug target. Cell Biol. Toxicol. 34, 87–91.

Xie, G.S., Hou, A.R., Li, L.Y., Gao, Y.N., Cheng, S.J., 2006. Aberrant p16 promoter methylation in cervical cancer is accompanied by reciprocal methylation changes of a CTCF-binding site. Hum. Mol. Genet. 15, 234–244.

Anderson, L., Mardis, E.R., Rasko, J.E.J., 2017. CTCF genetic alterations in endometrial carcinoma are pro-tumorigenic. Oncogene 36, 4100–4110.

Mendez-Catala, C.F., Grettan, S., Vostrov, A., Pugacheva, E., Farrar, D., Itu, O., Docoquier, F., Kita, G.X., Murrell, A., Lobanenkov, V., 2013. A novel mechanism for CTCF in the epigenetic regulation of Bax in breast cancer cells. Neoplasia 15, 898–912.

Mustafa, M., Lee, J.Y., Kim, M.H., 2015. CTCF negatively regulates HOXA10 expression in breast cancer cells. Biochem. Biophys. Res. Commun. 467, 828–834.

Nakahashi, H., Kieffer Kwon, K.R., Resch, W., Vian, L., Dose, M., Starvesa, D., Hakim, O., Pueett, N., Nelson, S., Yamane, A., Qian, J., Dubois, W., Welsh, S., Phair, R.D., Pugh, B.F., Lobanenkov, V., Hager, G.L., Casellas, R., 2013. A genome-wide map of CTCF multivalency redefines the CTCF code. Cell Rep., 3, 1678–1689.

Singh, S., Shrivastava, A.K., 2017. In silico characterization and transcriptomic analysis of nif family genes from Anabaena sp. PCC7120. Cell Biol. Toxicol. 33, 467–482.

Szalaj, P., Plewczynski, D., 2018. Three-dimensional organization and dynamics of the genome. Cell Biol. Toxicol. 34, 381–404.

Takai, D., Gonzales, F.A., Tsai, Y.C., Thayer, M.J., Jones, P.A., 2001. Large scale mapping of methylocytosines in CTCF-binding sites in the human H19 promoter and aberrant hypermethylation in human bladder cancer. Hum. Mol. Genet. 10, 2619–2626.

Terabayashi, T., Hanada, K., 2018. Genome instability syndromes caused by impaired DNA repair and aberrant DNA damage responses. Cell Biol. Toxicol. 34, 337–350.

Tiffen, J.C., Bailey, C.G., Marshall, A.D., Metierre, C., Wang, X., Watson, S.L., Holst, J., Rasko, J.E., 2013. The cancer-testis antigen BORIS phenocopies the tumor suppressor CTCF in normal and neoplastic cells. Int. J. Cancer 133, 1603–1613.

Ullner, G.A., Yu, T.H., Li, H., Yu, J.F., Yao, X.M., Wang, Q., Watson, S.L., Holst, J., Rasko, J.E., 2013. The cancer-testis antigen BORIS phenocopies the tumor suppressor CTCF in normal and neoplastic cells. Int. J. Cancer 133, 1603–1613.

Wang, X. 2018. Clinical trans-omics: an integration of clinical phenomes with molecular multiomics. Cell Biol. Toxicol., 34, 163–166.

Wu, D., Wang, X., Sun, H. 2018. The role of mitochondria in cellular toxicity as a potential drug target. Cell Biol. Toxicol. 34, 87–91.

Xie, G.S., Hou, A.R., Li, L.Y., Gao, Y.N., Cheng, S.J., 2006. Aberrant p16 promoter hypermethylation in bronchial mucosa in a biomarker for the early detection of lung cancer. Chin. Med. J. (Engl.) 119, 1469–1472.

Yeh, A., Wei, M., Golub, S.B., Yamashiro, D.J., Murty, V.V., Tycko, B., 2002. Chromosome arm 16q in Wilms tumors: unbalanced chromosomal translocations, loss of heterozygosity, and assessment of the CTCF gene. Genes Chromosom. Cancer 35, 156–163.

Zhang, R., Burke, L.J., Rasko, J.E., Lobanenkov, V., Renkawitz, R., 2004. Dynamic association of the mammalian insulator protein CTCF with centromeres and the midbody. Exp. Cell Res. 294, 86–93.