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Somatic deletion of *KDM1A/LSD1* gene is associated to advanced colorectal cancer stages

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ABSTRACT

Aims *KDM1A/LSD1* and *ZNF217* are involved in a protein complex that participates in transcriptional regulation. *ZNF217* has been analysed in numerous cancers and its amplification has been associated with advanced stages of disease; however, a similar role for *KDM1A/LSD1* has not been uncovered. In this study, we estimated the number of *KDM1A/LSD1* and *ZNF217* gene copies in tissue samples from patients diagnosed with colorectal cancer (CRC), as well as its association with clinicopathological features in patients with CRC.

Methods Paraffin-embedded tumour samples from 50 patients with CRC with a histopathological diagnosis of CRC were included. The number of copies of *KDM1A/LSD1* and *ZNF217* genes was determined by fluorescence in situ hybridisation (FISH). We also analysed the association between copy numbers of selected genes and clinicopathological data based on multivariate analysis.

Results Deletion of the *KDM1A/LSD1* gene occurred in 19 samples (38%), whereas *ZNF217* gene amplification was identified in 11 samples (22%). We found a significant association between lymph node metastasis or advanced tumour stage and *KDM1A/LSD1* gene deletion (p value=0.0003 and p value=0.011, respectively).

Conclusions *KDM1A/LSD1* gene deletion could be considered a novel prognostic biomarker of late-stage CRC.

INTRODUCTION

Worldwide, colorectal cancer (CRC) is the third most common neoplasm and the second leading cause of death related to cancer.¹ Environmental, epigenetic and genetic factors have been associated with this disease.² Molecular changes found in CRC can be classified into three main groups including microsatellite instability, CpG island methylator phenotype and chromosomal instability. The latter explains most CRC cases and is characterised by gains and losses of whole or partial chromosomes that could result in gene copy number variations.³

The chromosomal instability found in patients with CRC often includes gains in chromosomes 5, 7, 8q, 13, 20 and X, as well as losses in 18, 17p, 14, 5q, 4 and 1p.⁴ It has been documented that a 20q gain is associated with adenoma-carcinoma progression due to the selection of genes that confer an advantage for survival and growth,⁵ whereas 1p deletion occurs in 50% of CRC cases

and is related to tumour progression and metastasis.^{6,7} The *KDM1A/LSD1* gene is located in 1p36.12 and encodes the lysine-specific demethylase 1A⁸; in turn, this protein is involved in different complexes and functions such as cellular proliferation and chromosome segregation.⁹ More specifically, *KDM1A* and the *ZNF217/CoREST/CtBP1* complex co-operate to repress transcriptional activity.¹⁰ The *ZNF217* gene, mapped to 20q13.2 and described as an oncogene, is frequently duplicated in CRC.¹¹ Further, augmented gene copy numbers or amplification usually correlates with increased expression.^{12,13} Since *ZNF217* gene amplification has been identified in approximately 60%–65% of patients with CRC, it has been proposed to be a biomarker for this disease and perhaps other human cancers.¹¹

The transcriptional repressor complex *KDM1A–ZNF217* regulates tumour suppressor genes and thus plays a critical role in cancer development. *KDM1A* functions in the repression of chromatin structure by demethylating lysine 4 of histone H3 (H3K4), but also promotes the activation of target genes through methyl modifications to lysine 9 of histone H3 (H3K9); whereas *KDM1A* recognises specific substrates that are added to the complex,^{14,15} *ZNF217* protein acts as a transcriptional regulator of the target genes.¹⁶

Even though the amplification of *ZNF217* has been well documented in CRC, the amplification status of *KDM1A/LSD1* in any cancer type is not known. The aim of this work was to evaluate gene copy number variation in *KDM1A/LSD1* and *ZNF217* genes and its association with clinicopathological features in patients with CRC.

MATERIALS AND METHODS

Patients and tumour samples

Tumour samples and medical records from patients evaluated at the Histopathology Service of Civil Hospitals “Dr. Juan I. Menchaca” and “Fray Antonio Alcalde” at Guadalajara, Jalisco, Mexico, between 2003 and 2012 were included.

Fifty paraffin-embedded tumour samples of CRC were selected with a histopathological diagnosis of colorectal adenocarcinoma obtained before the patients received chemotherapy and/or radiation. Clinicopathological data were reviewed, such as age, sex, tumour location, tumour, node, metastases (TNM) stage, lymph node metastasis

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Figure 1 Fluorescence in situ hybridisation (FISH) results for the *KDM1A/LSD1* (A–C) and *ZNF217* (D–F) genes in patients with colorectal cancer (CRC). Image (A) shows the cells stained with DAPI; (B–C) gene deletion is interpreted for (B) chromosome 1 centromere signals in red versus (C) green signals for the *KDM1A/LSD1* gene. In addition, image (D) shows DAPI counterstaining; (E–F) gene amplification is considered according to (E) 20pter control signals in green versus (F) red signals of the *ZNF217* gene. Original magnification $\times 100$ using an Axiolmager. A1 epifluorescence microscope (Carl Zeiss, Jena, Germany). Uneven illumination was corrected using a control image as described by Marty.⁴¹

and distant metastasis. This work was a cross-sectional retrospective study.

Deparaffined and pretreatment samples

From sections comprising tumour cells that were defined histologically, 5 μ m thick slices were cut and fixed on slides for fluorescence in situ hybridisation (FISH) analysis. Briefly, slides were heated at 58°C overnight on a HYBrite TM (VYSIS, Downers Grove, Illinois, USA), run through a series of three steps in Citrisolv clearing agent (FISHER brand) for 10 min each, incubated twice in 100% ethanol for 5 min each at room temperature and left to dry. Slides were then deparaffined with the pretreatment kit I (2J02-32 Abbott).

Interphase FISH

FISH analysis was performed according to manufacturer's protocol by co-hybridising the sample and probes at 75°C in the HYBrite device. We used a probe that included the BAC clone RP11-152I19 (1p36.12, NCBI36/hg18, CHR1:23184031–23355672; Empire Genomics, USA) labelled with 5-fluorescein fluorophore and the reference CEP 1 ALPHA spectrum orange probe (D1Z5, Abbott Molecular 06J39-036, USA); these probes identify 100% of *KDM1A/LSD1* gene sequences and the chromosome 1 centromere (p11.1q11.1), respectively. We also used the *ZNF217*/1061L1 (Cytocell LPS 005, UK) specific probe for *ZNF217* in 20q13.2 (spectrum green) coupled with the reference probe 1061L1 located in 20pter (spectrum red). FISH signals were analysed using an Axiolmager.A1 epifluorescence microscope (Carl Zeiss, Jena, Germany) equipped with filters for DAPI (4',6-diamidino-2-phenylindole), FITC (fluorescein isothiocyanate) and Texas Red.

FISH interpretation

Two investigators (RR-R and MLA-M) evaluated 100 non-overlapping interphase cells for each case. The ratio of gene deletion or amplification was assessed by dividing the total number of target gene probe signals by the total number of control probe signals. A ratio value ≤ 0.85 was considered a gene deletion,¹⁷

Table 1 Clinical and histopathological characteristics of patients with colorectal cancer

	N (%)
Age	
Over 50	38 (76)
Under 50	12 (24)
Sex	
Female	22 (44)
Male	28 (56)
Tumour location	
Colon	38 (76)
Rectum	12 (24)
TNM stage	
I–II	26 (52)
III–IV	24 (48)
Lymph node metastasis	15 (30)
Distant metastasis	9 (18)

TNM, tumour/node/metastasis staging system.

whereas a ratio value ≥ 2 was established as gene amplification.¹⁸ Additionally, apparent aneuploidies of chromosome 1 and 20 were identified when the signal proportion of the gene target versus the control probes was different from 2:2. According to Kuhn *et al*,¹⁹ we interpreted these findings as polysomy (trisomy, tetrasomy or more) whenever ≥ 3 copies of the control probe were found in at least 40% of the cells analysed.

Statistical analysis

Statistical packages for data analysis were IBM SPSS Statistics Base V.22.0 and Microsoft Excel 2010, and we used Pearson correlation analysis to test for any association between FISH results for *KDM1A/LSD1* and *ZNF217* genes and CRC characteristics. To evaluate predictive factors, a multivariate analysis of age, sex, tumour location, TNM stage, lymph node metastasis and distant metastasis was performed. A $p < 0.05$ was considered statistically significant.

RESULTS

The 50 patients with CRC had a mean age of 61 years (range 28–89). Their characteristics are shown in [table 1](#).

KDM1A analysis

A loss of the *KDM1A/LSD1* gene was detected in 38% ($n=19$) of the analysed samples ([figure 1](#)). With respect to clinical and histopathological characteristics, this deletion was only associated with metastasis to the lymph nodes ($p=0.0003$) and TNM stage III–IV ($p<0.011$). In addition, chromosome 1 gains were observed in 64% ($n=32$) of samples.

ZNF217 analysis

The expected amplification of *ZNF217* was observed in only 22% ($n=11$) of cases ([figure 1](#)), but it was not associated with any clinical or histopathological characteristics. Chromosome 20 polysomy was observed in 66% ($n=33$) of CRC cases.

The concurrence of *KDM1A/LSD1* gene deletion and *ZNF217* gene gain (either by gene amplification and/or polysomy) was found in 34% ($n=17$) of cases ([table 2](#)) and was associated with metastasis to the lymph node ($p<0.0007$) and TNM stage III–IV ($p<0.025$).

Copy number variation of *KDM1A/LSD1* and *ZNF217* was assessed in 43 of 50 CRC patient samples. The corresponding

Table 2 Findings for *KDM1A/LSD1* and *ZNF217* gene copy number variation in patients with colorectal cancer

Patient	Sex	Age	<i>KDM1A/LSD1</i> deletion	<i>ZNF217</i> amplification	Chr 1 polysomy	Chr 20 polysomy
2	F	62	+		+	+
3	M	62				
4	M	76	+			
6	M	48		+		
7	F	47	+			+
8	F	67	+		+	+
9	F	69		+	+	+
10	F	55				+
11	M	78			+	+
12	F	89		+	+	+
13	M	78			+	+
14	F	62		+	+	+
15	M	84				+
16	F	47				+
18	F	69			+	+
19	F	75	+		+	+
20	M	70	+	+	+	+
21	M	41		+	+	
22	F	82			+	+
23	F	63	+		+	+
24	M	77	+		+	
25	M	53	+		+	+
26	M	41	+		+	+
27	M	35	+		+	+
28	M	52	+	+	+	
29	F	76	+	+	+	+
31	M	58	+		+	+
32	M	56	+		+	+
33	F	57			+	
34	F	48	+		+	+
35	M	50				+
36	M	71			+	+
37	M	38			+	+
39	M	64		+	+	
40	F	69			+	+
42	M	65	+			+
44	F	34			+	+
45	M	55		+	+	
46	F	41				+
47	M	63	+		+	+
48	F	62			+	+
49	F	85				+
50	M	52	+	+	+	

+, positive alteration; F, female; M, male.

data including polysomies of chromosomes 1 and 20 are shown in table 2. It was found that 32% (n=16) of patients harboured both *KDM1A/LSD1* gene deletion and chromosome 1 polysomy. Regarding the *ZNF217* gene, only 10% (n=5) of the patient samples exhibited chromosome 20 polysomy and gene amplification.

DISCUSSION

KDM1A and *ZNF217* proteins are components of transcriptional complexes that positively modulate or co-repress the

expression of target genes including some related to carcinogenesis.^{10 14} Actually, Sehwat *et al*²⁰ demonstrated that *KDM1A* in association with *ZNF217* stimulates prostate cancer cell survival, through activation of the cell cycle and embryonic stem cell genes that are enriched in lethal prostate tumours. This supports the fundamental role of both proteins in transcriptional regulation during cancer.

In the present assessment of copy number variations in *KDM1A* and *ZNF217*, we identified *ZNF217* amplification in only 22% of CRC samples. Gene amplification has been associated with poor prognosis in different types of cancer.¹⁶ In CRC, several studies have reported that *ZNF217* gene amplification is associated with increased metastatic potential,²¹ poorer survival¹¹ and more aggressive clinical behaviour,²² whereas a poor response or resistance to treatment with 5-fluorouracil was found to be related to CRC microsatellite instability.²³ However, we did not find any association between *ZNF217* amplification and clinical or histopathological data. This lack of association could be ascribed to the lower ratio of gene amplification as compared with frequencies of 41%–100% found in studies supporting such associations^{11 21 22}; however, it must be noted that Hidaka *et al*²¹ classified samples according to liver metastasis and that Postma *et al*²² analysed flat colorectal carcinomas, in addition to the fact that in both studies, the sample size was smaller than ours. Similarly, Huang *et al*²⁴ did not identify prognostic significance for *ZNF217* amplification based on that observed in 31% of 68 patients with ovarian clear cell carcinoma.

Regarding *KDM1A/LSD1* gene copy variation, we found gene deletion in 38% of patients. Even though no other marker on chromosome 1p was analysed and the *KDM1A/LSD1* gene is located on 1p36.12, our findings could still be related to the loss of chromosome 1p, since this chromosomal region is deleted in up to 50% of colorectal carcinomas.⁷ Chromosome 1p36 contains several candidate tumour suppressor genes such as *CHD5*, *CAMTA1*, *KIF1B* and *CASZ1*, which are related to the modulation of chromatin structure, transcription (via interactions with histones), poor prognosis in cancer, apoptosis, cell migration, cell proliferation and tumorigenicity.²⁵ Nevertheless, the relevance of *KDM1A/LSD1* might be greater due to its participation in several cellular processes such as histone and DNA methylation,²⁶ cell differentiation,²⁷ cell proliferation,²⁸ chromatid segregation during mitosis²⁹ and epithelial-mesenchymal transition (EMT), a phenomenon well known to facilitate metastasis.³⁰ Indeed, *KDM1A* interacts with *SNAIL1* to recruit the *KDM1A* corepressor complex, which in turn leads to the H3K4me2 demethylation of E-cadherin-associated active promoters and the downregulation of *SNAIL1* and/or E-cadherin, which promotes cell motility and ultimately EMT.^{31 32}

By including results of *KDM1A/LSD1* gene deletion and clinicopathological data in a multivariate analysis, we identified a relationship between lymph node metastasis or advanced tumour stages and *KDM1A/LSD1* gene deletion (p values=0.0003 and 0.011, respectively). Hence, this deletion could be a useful biomarker for the late stages of cancer. In accordance, Wang *et al*³³ showed that *KDM1A* protein is part of the Mi-2/nucleosome remodelling and deacetylase corepressor complex and performed in vitro assays to assess the effect of this protein on breast cancer cells. These authors found that the overexpression of wild-type *KDM1A* led to a threefold decrease in cell invasion, whereas knockdown increased cell invasiveness by approximately fivefold. Moreover, Wang *et al* obtained the same results after analysing breast cancer metastasis in mice, wherein lung metastasis was suppressed in tumours with *KDM1A* overexpression and metastatic spread was found in the absence of *KDM1A*.

Altogether, these data suggest that *KDM1A/LSD1* gene suppression is key during metastasis. The apparent discrepancy between this conclusion and the previously annotated role for *KDM1A* in cancer, especially the association between *KDM1A* overexpression and poor prognosis and the proliferation and invasion of several neoplasms, such as lung, liver, oesophagus, and colon cancers (15), can be resolved if we consider the genetic background of the cells and the effect of environmental signals on *KDM1A*.³⁴

Based on the analysis of gene copy numbers, we also inferred aneuploidies of chromosome 1 and 20 from the FISH patterns for gene and control signals. We found 32 (64%) and 33 cases (66%) with gains of chromosome 1 and 20, respectively. Comparable polysomies, mainly trisomies and tetrasomies, are common in cancer cells.³⁵ Although aneuploidies, and specifically trisomies, can suppress tumour growth in the short term, they improve the long-term advantages of premalignant cells and generate a destabilising effect that contributes to the aggressive growth of tumours.³⁶ It seems that aneuploidy, found in ~85% of different cancers,³⁷ results in heterogeneity and facilitates the adaptation of cancer cells via the acquisition of advantageous features.³⁸ In CRC, aneuploidy is observed in advanced stages probably as a consequence of genetic alterations in diploid or polyploid cells.^{39,40}

Despite the fact that simultaneous *KDM1A/LSD1* deletions and *ZNF217* gains occurred in 34% of patients and was associated with metastasis to the lymph node ($p < 0.0007$) and TNM stage III–IV ($p < 0.025$), this was not found to comprise a synergistic effect of gene copy number variation, since *ZNF217* gains and clinicopathological characteristics were not associated in patients with CRC. Since small sample size is a limitation of this study, the lack of association between *ZNF217* amplification and CRC could also be related to the low number of advanced-stage tumour samples analysed. Notwithstanding, given the colocalisation of *KDM1A/LSD1* and *ZNF217* proteins in the transcriptional repression protein complex, the findings of deletion and amplification of *KDM1A/LSD1* and *ZNF217* genes could still be related to CRC progression, and further studies based on CRC patients with advanced tumour stages or metastasis are required.

In conclusion, our results show that *KDM1A/LSD1* gene deletion is associated with lymph node metastasis and advanced stages of CRC and could be considered a biomarker of prognostic value in this common malignancy. Synergism between *KDM1A/LSD1* gene losses and *ZNF217* gene gains has also emerged as possible event relevant to the progression of CRC. Finally, to our knowledge, this is the first report to analyse *KDM1A/LSD1* and *ZNF217* copy numbers and their association with clinicopathological data in patients with CRC.

Take home messages

- Deletion of the *KDM1A/LSD1* gene is associated with lymph node metastasis and advance tumour stages in patients with CRC.
- *KDM1A/LSD1* gene deletion could be a useful biomarker of late-stage colorectal carcinomas.
- Deletion of *KDM1A/LSD1* and amplification of *ZNF217* genes could exert a synergistic effect on the progression of CRC.

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Competing interests None declared.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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REFERENCES

- Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394–424.
- Obuch JC, Ahnen DJ. Colorectal cancer: genetics is changing everything. *Gastroenterol Clin North* 2016;45:459–76.
- Tariq K, Ghias K, Tariq K, et al. Colorectal cancer carcinogenesis: a review of mechanisms. *Cancer Biol Med* 2016;13:120–35.
- Migliore L, Mighelli F, Spisni R, et al. Genetics, cytogenetics, and epigenetics of colorectal cancer. *J Biomed Biotechnol* 2011;2011:1–19.
- Nguyen HT, Duong HQ. The molecular characteristics of colorectal cancer: implications for diagnosis and therapy. *Oncol Lett* 2018;16:9–18.
- Thorstensen L, Qvist H, Heim S, et al. Evaluation of 1p losses in primary carcinomas, local recurrences and peripheral metastases from colorectal cancer patients. *Neoplasia* 2000;2:514–22.
- Knösel T, Schlüns K, Stein U, et al. Chromosomal alterations during lymphatic and liver metastasis formation of colorectal cancer. *Neoplasia* 2004;6:23–8.
- NCBI. Gene [Internet]. Bethesda (MD): National Center for Biotechnology Information (US), National Library of Medicine, 2007. Available: <https://www.ncbi.nlm.nih.gov/gene/23028> [Accessed 22 Feb 2019].

9. Kozub MM, Carr RM, Lomberg GL, *et al.* Lsd1, a double-edged sword, confers dynamic chromatin regulation but commonly promotes aberrant cell growth. *F1000Res* 2016;2017.
10. Banck MS, Li S, Nishio H, *et al.* The ZNF217 oncogene is a candidate organizer of repressive histone modifiers. *Epigenetics* 2009;4:100–6.
11. Rooney PH, Boonsong A, McFadyen MCE, *et al.* The candidate oncogene ZNF217 is frequently amplified in colon cancer. *J Pathol* 2004;204:282–8.
12. Bagci O, Kurtgöz S. Amplification of cellular oncogenes in solid tumors. *N Am J Med Sci* 2015;7:341–6.
13. Quinlan KG, Verger A, Yaswen P, *et al.* Crossley M: amplification of zinc finger gene 217 (ZNF217) and cancer: when good fingers go bad. *Biochim Biophys Acta* 1775;2007:333–40.
14. Thillainadesan G, Iovic M, Loney E, *et al.* Genome analysis identifies the p15INK4B tumor suppressor as a direct target of the ZNF217/CoREST complex. *Mol Cell Biol* 2008;28:6066–77.
15. Nagasawa S, Sedukhina AS, Nakagawa Y, *et al.* Lsd1 overexpression is associated with poor prognosis in basal-like breast cancer, and sensitivity to PARP inhibition. *PLoS One* 2015;10:e0118002.
16. Cohen PA, Donini CF, Nguyen NT, *et al.* The dark side of ZNF217, a key regulator of tumorigenesis with powerful biomarker value. *Oncotarget* 2015;6:41566–81.
17. Pinkham MB, Telford N, Whitfield GA, *et al.* Fishing tips: what every clinician should know about 1p19q analysis in gliomas using fluorescence in situ hybridisation. *Clin Oncol* 2015;27:445–53.
18. Geppert C-I, Rümmele P, Sarbia M, *et al.* Multi-colour fish in oesophageal adenocarcinoma—predictors of prognosis independent of stage and grade. *Br J Cancer* 2014;110:2985–95.
19. Kuhn E, Bahadırli-Talbot A, Shih I-M. Frequent CCNE1 amplification in endometrial intraepithelial carcinoma and uterine serous carcinoma. *Mod Pathol* 2014;27:1014–9.
20. Sehwat A, Gao L, Wang Y, *et al.* Lsd1 activates a lethal prostate cancer gene network independently of its demethylase function. *Proc Natl Acad Sci U S A*;20181:E4179–88.
21. Hidaka S, Yasutake T, Takeshita H, *et al.* Differences in 20q13.2 copy number between colorectal cancers with and without liver metastasis. *Clin Cancer Res* 2000;6:2712–7.
22. Postma C, Hermesen M, Coffa J, *et al.* Chromosomal instability in flat adenomas and carcinomas of the colon. *J Pathol* 2005;205:514–21.
23. Lassmann S, Weis R, Makowiec F, *et al.* Array CGH identifies distinct DNA copy number profiles of oncogenes and tumor suppressor genes in chromosomal- and microsatellite-unstable sporadic colorectal carcinomas. *J Mol Med* 2007;85:293–304.
24. Huang H-N, Huang W-C, Lin C-H, *et al.* Chromosome 20q13.2 ZNF217 locus amplification correlates with decreased E-cadherin expression in ovarian clear cell carcinoma with PI3K-Akt pathway alterations. *Hum Pathol* 2014;45:2318–25.
25. Henrich K-O, Schwab M, Westermann F. 1P36 tumor Suppression—A matter of dosage? *Cancer Res* 2012;72:6079–88.
26. Wang J, Hevi S, Kurash JK, *et al.* The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat Genet* 2009;41:125–9.
27. Amente S, Lania L, Majello B. The histone LSD1 demethylase in stemness and cancer transcription programs. *Biochim Biophys Acta* 1829;2013:981–6.
28. Scoumanne A, Chen X. The lysine-specific demethylase 1 is required for cell proliferation in both p53-dependent and -independent manners. *J Biol Chem* 2007;282:15471–5.
29. Lv S, Bu W, Jiao H, *et al.* Lsd1 is required for chromosome segregation during mitosis. *Eur J Cell Biol* 2010;89:557–63.
30. Ambrosio S, Sacc CD, Majello B. Epigenetic regulation of epithelial to mesenchymal transition by the lysine-specific demethylase LSD1/KDM1A. *Biochim Biophys Acta Gene Regul Mech* 1860;2017:905–10.
31. Lin T, Ponn A, Hu X, *et al.* Requirement of the histone demethylase LSD1 in SNAI1-mediated transcriptional repression during epithelial-mesenchymal transition. *Oncogene* 2010;29:4896–904.
32. Ismail T, Lee H-K, Kim C, *et al.* KDM1A microenvironment, its oncogenic potential, and therapeutic significance. *Epigenetics Chromatin* 2018;11:33.
33. Wang Y, Zhang H, Chen Y, *et al.* Lsd1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. *Cell* 2009;138:660–72.
34. Hino S, Kohrogi K, Nakao M. Histone demethylase LSD1 controls the phenotypic plasticity of cancer cells. *Cancer Sci* 2016;107:1187–92.
35. Mitelman F, Johansson B, Mertens F. Mitelman database of chromosome aberrations and gene fusions in cancer, 2019. Available: <http://cgap.nci.nih.gov/Chromosomes/Mitelman> [Accessed 1 Apr 2019].
36. Sheltzer JM, Ko JH, Replogle JM, *et al.* Single-chromosome gains commonly function as tumor suppressors. *Cancer Cell* 2017;31:240–55.
37. Weaver BAA, Cleveland DW. Does aneuploidy cause cancer? *Curr Opin Cell Biol* 2006;18:658–67.
38. Cosenza MR, Krämer A. Centrosome amplification, chromosomal instability and cancer: mechanistic, clinical and therapeutic issues. *Chromosome Res* 2016;24:105–26.
39. Sugai T, Uesugi N, Nakamura S-ichi, *et al.* Evolution of DNA ploidy state and DNA index in colorectal adenomas and carcinomas using the crypt isolation technique: new hypothesis in colorectal tumorigenesis. *Pathol Int* 2003;53:154–62.
40. De Angelis PM, Stokke T, Beigi M, *et al.* Chromosomal 20q gain in the DNA diploid component of aneuploid colorectal carcinomas. *Int J Cancer* 2007;120:2734–8.
41. Marty GD. Blank-field correction for achieving a uniform white background in brightfield digital photomicrographs. *Biotechniques* 2007;42:716–20.