A KHDC3L mutation resulting in recurrent hydatidiform mole causes genome-wide DNA methylation loss in oocytes and persistent imprinting defects post-fertilisation

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Abstract

Maternal effect mutations in the components of the subcortical maternal complex (SCMC) of the human oocyte can cause early embryonic failure, gestational abnormalities and recurrent pregnancy loss. Enigmatically, they are also associated with DNA methylation abnormalities at imprinted genes in conceptuses: in the devastating gestational abnormality biparental complete hydatidiform mole (BiCHM) or in multi-locus imprinting disease (MLID). However, the developmental timing, genomic extent and mechanistic basis of these imprinting defects are unknown. The rarity of these disorders and the possibility that methylation defects originate in oocytes have made these questions very challenging to address.

Single-cell bisulphite sequencing (scBS-seq) was used to assess methylation in oocytes from a patient with BiCHM identified to be homozygous for an inactivating mutation in the human SCMC component KHDC3L. Genome-wide methylation analysis of a preimplantation embryo and molar tissue from the same patient was also performed.

High-coverage scBS-seq libraries were obtained from five c.1A>G oocytes, which revealed a genome-wide deficit of DNA methylation compared with normal human oocytes. Importantly, germline differentially methylated regions (gDMRs) of imprinted genes were affected similarly to other sequence features that normally become methylated in oocytes, indicating no selectivity towards imprinted genes. A range of methylation losses was observed across genomic features, including gDMRs, indicating variable sensitivity to defects in the SCMC. Genome-wide analysis of a pre-implantation embryo and molar tissue from the same patient showed that following fertilisation methylation defects at imprinted genes persist, while most non-imprinted regions of the genome recover near-normal methylation post-implantation.

We show for the first time that the integrity of the SCMC is essential for de novo methylation in the female germline. These findings have important implications for understanding the role of the SCMC in DNA methylation and for the origin of imprinting defects, for counselling affected families, and will help inform future therapeutic approaches.

Epigenetics, DNA methylation, Genomic imprinting, Single-cell analysis, Oocytes, Embryos

B

Background

The subcortical maternal complex (SCMC), a multi-protein structure of the mammalian oocyte, orchestrates a number of essential cellular processes during the oocyte-to-embryo transition, such as spindle assembly, chromosome alignment and symmetric cell division in cleavage-stage embryos [1, 2]. In humans, mutations in SCMC proteins cause various developmental abnormalities, including early embryonic arrest and reproductive failure [3 9]. A recurrent, but unexplained, finding is abnormalities in genomic imprinting. Maternal recessive mutations in NLRP7 and KHDC3L, both indicated to encode SCMC components [3, 4, 10, 11], are the predominant cause of biparental, complete hydatidiform mole (BiCHM; also referred to as recurrent, familial hydatidiform mole; OMIM 231090 and 614293), a rare gestational abnormality characterised by trophoblast overgrowth and absence of embryo development. NLRP7 or KHDC3L mutations are found in the majority of BiCHM cases (NLRP7 ~ 75%, KHDC3L 5 10%) and are associated with widespread loss of methylation (LoM) of germline differentially methylated regions (gDMRs) of imprinted genes in molar tissue [3, 4, 12, 13]. In addition, mutations in other SCMC components, including PADI6, OOEP, NLRP5 and NLRP2, have been described in single imprinting syndromes or multi-locus imprinting disturbance (MLID), and PADI6 has been associated with molar pregnancies [5, 8, 14]. However, the molecular aetiology of BiCHM and MLID is obscure, as connections between the SCMC under the oocyte plasma membrane and the nuclear DNA methylation machinery have not been defined. This lack of molecular understanding prevents a meaningful development of therapeutic approaches or satisfactory counselling of affected families. Mouse models have thus far not been informative, because there are no direct homologues of KHDC3L or NLRP7, and because maternal effect mutations in Nlrp5/Mater, Ooep/Floped and Tle6 lead to very early developmental arrest [1, 15, 16]. Analysis of a mouse Nlrp2 knockout, which is compatible with development to term but with reduced fertility, has implicated a defect in methylation maintenance of imprinted genes post-fertilisation. It was shown that localisation of the maintenance DNA methyltransferase DNMT1 with the SCMC is disrupted in Nlrp2 knockout oocytes while DNMT3A, the predominant methyltransferase responsible for de novo methylation in the oocyte, retains normal chromosome association [17]. However, while mid-gestation embryos and neonates from Nlrp2-deficient oocytes exhibit limited methylation alterations of some imprinted genes, there is no generalised LoM of imprints typical of molar tissue [17

patient with a sporadic case of androgenic complete hydatidiform mole (AnCHM). DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen), and contamination of molar tissue was tested by Chromoquant QF-PCR kit (CyberGene AB).

Oocytes were obtained voluntarily from patient D at the IVF centre of the Ghadir Mother and Child Hospital affiliated to Shiraz University of Medical Sciences with signed informed consent of the patient and her husband and the approval of the Ethics Committee of Shiraz University of Medical Sciences (ethics codes: IR.sums.rec.1395.S718 for oocyte retrieval and IR.sums.rec.1396.S779 for embryo production). Mature oocytes were obtained after ovarian stimulation using a standard gonadotropin-releasing hormone (GnRH) antagonist protocol. Oocytes were collected in G-IVF plus (Vitrolife) and cleaned in G-MOPS (Vitrolife) supplemented with 80 IU/ml hyaluronidase (HYASE-10X, Vitrolife). Out of nine oocytes, seven were collected for subsequent scBS-seq analysis. Intracytoplasmic sperm injection (ICSI) was performed followed by 6 days embryo culture with the two remaining oocytes, resulting in one embryo, which was collected in $< 5\,\mu l$ RLT buffer for

DNA methylation of single oocytes was assessed using WGBS according to the single-cell adaptation (scBS-seq) of the post-bisulphite adaptor tagging (PBAT) method as previously described [21, 28]. The PBAT protocol was also employed to analyse DNA methylation of the embryo, using a slightly adapted method for bulk samples as de-

using a Shapiro-Wilk normality test. DNA methylation differences between KHDC3L$^{c.1A>G}$ mole and control placenta were analysed with a Wilcoxon signed-rank test. Differences between KHDC3L$^{c.1A>G}$ and control oocytes in global CpG methylation and methylation of ZFP57-binding sites were determined using an unpaired

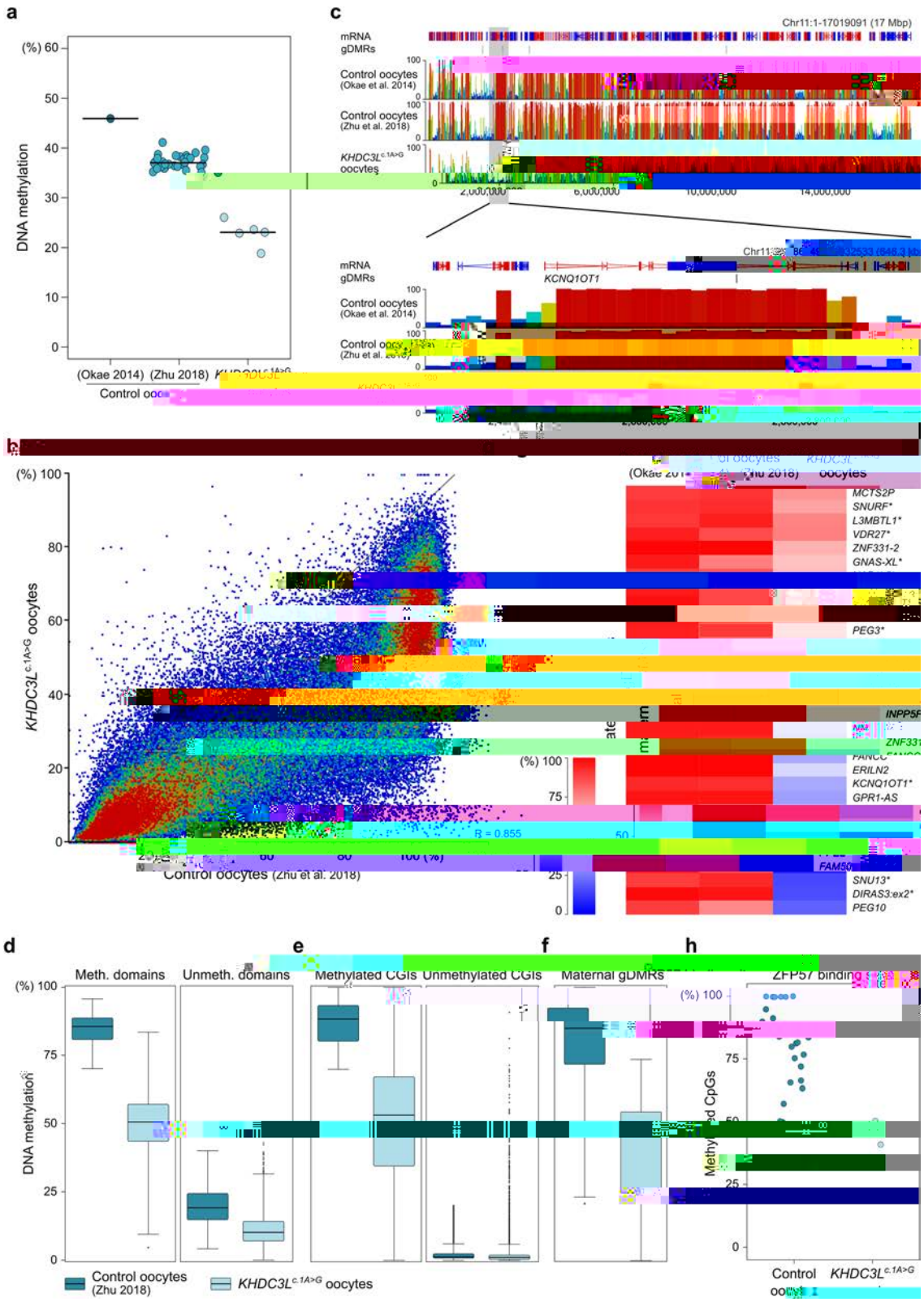
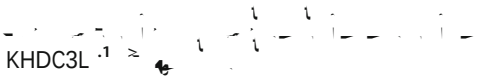


Fig. 2 (See legend on next page.)

exhibited a reduced rate of methylation in KHDC3L$^{c.1A>G}$ oocytes, of a similar magnitude to the loss at gDMRs (Fig. 2h).



The remaining 2 MII oocytes were subject to intracytoplasmic sperm injection from which 1 embryo developed in vitro until the late morula/early blastocyst stage at day 6 (Additional file 3: Figure S8A) when it was collected for methylation analysis. Despite being developmentally less advanced than normal blastocysts, the KHDC3L$^{c.1A>G}$ embryo had reduced methylation in comparison with con-

therefore manifest more severe methylation defects than an embryo from which molar tissue could arise.

How could a defect in the SCMC or in the protein components impair DNA methylation establishment in the oocyte? The mechanisms of de novo methylation in oocytes are best understood in the mouse, benefitting from genetic manipulations [18]. De novo methylation takes place on a genome largely demethylated after specification of primordial germ cells, in the latter stages of oocyte growth (secondary to antral follicle stage), and culminates in a distinctive methylation landscape with methylation preferentially over expressed gene bodies [21, 30, 35, 38]. Imprinted gDMR methylation is part of this generalised transcription-dependent mechanism [35, 39, 40]. Successful methylation establishment involves the interplay of several nuclear processes. In mice, the required de novo methyltransferase proteins DNMT3A and DNMT3L become abundant in oocytes concomitant with the onset of methylation [41]. Genomic recruitment of DNMT3A/DNMT3L is assumed to depend upon an appropriate chromatin state. DNA methylation coincides with domains of enrichment of histone 3 lysine 36 trimethylation (H3K36me3) over expressed genes, deposited by the unique H3K36me3 methyltransferase SETD2 [42]. Conversely, the histone mark H3K4me3 conventionally enriched at active promoters is antagonistic to DNMT3A/3L recruitment and activity [43, 44], and removal of H3K4 methylation at gDMRs requires

transcription-coupled nucleosome remodelling and/or activity of H3K4 demethylases such as KDM1B [35, 45, 46]. The normal methylation pattern also depends upon the exclusion of DNMT1 and its auxiliary protein UHRF1 from the nucleus, which otherwise leads to methylation of intergenic regions: this nuclear exclusion depends on the protein STELLA/PGC7 [47]. How could the global effect on methylation we observe in KHDC3L$^{c.1A>G}$ oocytes be explained? Considering the major role of transcription in specifying methylation in oocytes, a global problem in transcription could lead to a generalised deficit in methylation; however, this seems unlikely, as a major effect on transcription sufficient to attenuate methylation to the magnitude observed would likely be incompatible with full development and maturation of the oocyte. RNA-seq analysis of KHDC3L$^{c.1A>G}$ oocytes would be required to determine whether transcription defects could account for the variable methylation loss across maternal gDMRs and other genomic features. Gross reductions in the abundance or nuclear localisation of some of the key players above, such as DNMT3A or SETD2, could also cause the effects observed. However, loss of SETD2 in mouse oocytes, in addition to abrogating gene body methylation, leads to substantial methylation gain in intergenic regions [42], which we do not observe in the KHDC3L$^{c.1A>G}$ oocytes. The involvement of STELLA in sequestering DNMT1/UHFR1 from the nucleus [47] demonstrates the importance of regulated s(e)[(D(r)7.(R1)-390.1(fr)18.e)(genero151.1(pl8(qu)13.3(es)16.3(te3-392a1.229.2(ed9.1(fr)18.51(.56Gan)-39Td6n)15

discovery of a primary oocyte defect will now focus attention on how the mechanism of methylation establishment is globally impaired by defects in the SCMC. It also has important implications for possible therapeutic interventions in patient oocytes; these would be very challenging and, if to be considered, would have to aim to restore de novo methylation during oocyte growth, perhaps by injection of KHDC3L cRNA into oocytes growing in in vitro follicle culture systems [59 61].

Supplementary information

accompanies this paper at https://doi.org/10.1186/s13073-019-0694-y.

Additional file 1 : **1** List of gDMR coordinates; list of ZFP57 binding sites in gDMRs; .xls, 25 KB.

Additional file 2 : **2** methylation

The patient and husband consented to publication of information reported in this study.

The authors declare that they have no competing interests.

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