Short Conceptual Overview

Andreas C. Lawaetz and Kristian Almstrup*

Involvement of epigenetic modifiers in the pathogenesis of testicular dysgenesis and germ cell cancer

DOI 10.1515/bmc-2015-0006
Received February 12, 2015; accepted May 10, 2015

Abstract: Testicular germ cell cancer manifests mainly in young adults as a seminoma or non-seminoma. The solid tumors are preceded by the presence of a non-invasive precursor cell, the carcinoma *in situ* cell (CIS), which shows great similarity to fetal germ cells. It is therefore hypothesized that the CIS cell is a fetal germ cell that has been arrested during development due to testicular dysgenesis. CIS cells retain a fetal and open chromatin structure, and recently several epigenetic modifiers have been suggested to be involved in testicular dysgenesis in mice. We here review the possible involvement of epigenetic modifiers with a focus on jumonji C enzymes in the development of testicular dysgenesis and germ cell cancer in men.

Keywords: epigenetic modifiers; germ cell cancer; jumonji C-domain containing; testicular dysgenesis.

Introduction

The vast majority of human testicular tumors are derived from germ cells and develop in young adult men between 17 and 45 years of age. The incidence of testicular germ cell tumors (TGCTs) has been increasing over several decades and is the most frequently found cancer among young adult men in most countries (1–3). It manifests either as a seminoma or non-seminoma, which are very different tumors in terms of both histology and treatment regimes. Seminomas are homogeneous tumors composed of mitotically dividing germ cells, whereas non-seminomas are heterogeneous tumors that may contain varying proportions of undifferentiated embryonal carcinoma, partially differentiated somatic tissues (teratoma) and extra-embryonic elements such as choriocarcinoma and yolk sac tumors (4). Both of these very different tumor types are, however, preceded by a symptomless and pre-invasive stage, where the common precursor cell of TGCTs, the so-called carcinoma *in situ* (CIS) cell, can be found *in situ* in normally arranged seminiferous tubules.

Development of testicular cancer

It is believed that the CIS is formed *in utero* as studies of protein markers [reviewed in (5)], global gene expression profiles of CIS cells (6–8) and microRNAs (9) have provided evidence that CIS cells are very similar to fetal germ cells (gonocytes). It is thus very likely that a CIS cell is a gonocyte that failed to mature during development as outlined in Figure 1. Like the fetal germ cells, the CIS cells express a large range of stemness genes, including *POU5F1 (OCT-3/4), NANOG, LIN28-A, DPPA4, DPPA5, KIT and TFAP2C* (6–8, 10–14) and is regarded as pluripotent or even totipotent (can give rise to both embryonic and extra-embryonic cell types).

Further transformation of CIS cells most likely occurs during adaptation to the changed endocrine environment of the peri- and post-pubertal testis, when the cells undergo polyploidisation (15) and acquire a characteristic pattern of genomic aberrations, including the gain of chromosome 12p (16, 17), often seen as the isochromosome 12p (18).

Our current hypothesis is that developmental arrest of gonocytes is caused by disruption of the endocrine

***Corresponding author: Kristian Almstrup,** University Department of Growth and Reproduction, Section GR-5064, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark,
e-mail: kristian@almstrup.net.
http://orcid.org/0000-0002-1832-0307
Andreas C. Lawaetz: University Department of Growth and Reproduction, Section GR-5064, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

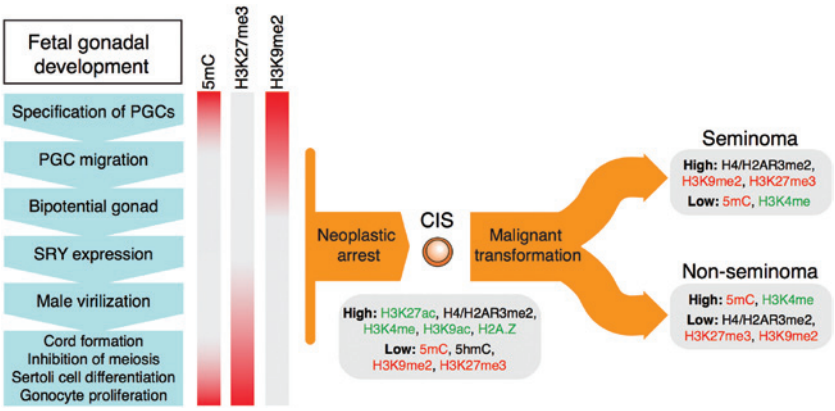

Figure 1: Schematic representation of the relationship between events of fetal gonadal development and epigenetic patterns observed in germ cells and in the pathogenesis of TGCT.
Epigenetic marks denoted with red and green are generally associated with repressive and active chromatin, respectively. Jumonji C domain-containing proteins play important regulatory roles during fetal gonadal development by regulating histone lysine methylation marks of, e.g. the *Sry* promoter in somatic gonadal cells. Histone lysine methylation marks are important regulatory set points in both germ and somatic cells during normal fetal gonadal development but also in the neoplastic and malignant transformation in the pathogenesis of TGCTs. CIS, carcinoma in situ; PGC, primordial germ cells.

environment in the testes (niche), which is comprised of somatic cells that secrete hormones and paracrine factors that stimulate male-specific germ cell maturation into spermatogonia (19, 20). This has recently been emphasized by the identification of DMRT1 and meiosis markers in pre-pubertal CIS cells indicative of a 'feminized' CIS cell (21).

Testicular dysgenesis syndrome

Observations of an increasing trend in the incidence of TGCTs and congenital reproductive tract malformations at the same time as a downward trend in semen quality and testosterone levels have led to the proposal that these conditions may represent a syndrome of disorders, a testicular dysgenesis syndrome (TDS) (22). Most of these disorders share common risk factors and are risk factors for each other, and all involve a potential disturbance in the development of the testes during fetal life. The aetiology of TDS is unclear, but the apparent rapid increase in male reproductive health problems during a few generations suggests that changes in lifestyle and/or environmental factors are more likely causes than genetic factors. Nevertheless, testicular cancer is known to be one of the most genetically predisposed cancers. Having an affected father or brother causes approximately a four- and nine-times increased risk, respectively (23), which is greater than most other common cancers. Genome-wide association studies have indicated that genetics can account for approximately 1/4 of the cases (24–29), and risk alleles include genes like *KITLG (SCF), DMRT1, SPRY4, and BAK1*, which have all been described as being involved in fetal gonadal and germ-cell development. Recently, pathway-based analyses of genome-wide association data have revealed that genes specifically involved in sex determination are associated with testicular cancer (30) and that many of the TGCT risk alleles also are risk alleles for TDS (31). Hence, the close relationship between sex determination, TDS and testicular cancer is genetically evident and is also reflected both histologically and clinically. However, among the risk alleles associated with TGCT, none of the jumonji C domain-containing genes reached significance. Histologically, TGCTs often coincide with dysgenetic tubules presenting spermatogenic arrest, micro-calcifications, poorly differentiated Sertoli cells, Sertoli-cell-only patterns, etc. (32, 33). Clinically, men with low sperm quality or reduced fertility have a three-fold increased risk of developing TGCTs (34, 35). Much evidence therefore points toward a close similarity in the aetiology of TDS and TGCT.

Epigenetics in the development of testicular cancer

The epigenetic state of CIS cells shows high similarity to the epigenetic patterns found in fetal germ cells (36). CIS

cells contain very low levels of DNA methylation when detected by immunohistochemical staining for 5-methyl-cytosine (5mC) (37), and apparently this unmethylated state is actively maintained by expression of APOBEC1 and base excision repair proteins MBD4, APEX1 and PARP1 (38). Another possible demethylation pathway, the conversion of 5mC to hydroxyl-methyl-cytosine (5hmC), was not found active in CIS cells as both 5hmC and the TET proteins, which convert 5mC to 5hmC, were not detected in CIS cells (38). Histone modifications H3K9me2 (Histone H3 dimethyl Lys9) and H3K27me3, both associated with a restrictive chromatin structure, are absent or found only at low levels in CIS cells, but H3K4me1, H3K4me2/3, H3K9ac, H3K27ac and the histone variant H2A.Z, which all are modifications associated with active and permissive chromatin structures, are abundantly present (36). Moreover, CIS cells show high levels of H4/H2AR3me2 along with expression of BLIMP1/PRMT5 (39), which are thought to repress *HOX* genes and somatic differentiation programs (40). Another possible way of regulating *HOX* genes is through repression by the polycomb group proteins as seen in human embryonic stem cells (41). This regulation is, however, dependent on H3K27me3 marks (42, 43), which as mentioned above are absent or found only at low levels in CIS cells. Moreover, the known H3K27me3 demethylases, UTX (KDM6A) and JMJD3 (KDM6B), which regulate *HOX* gene expression in human embryonic teratocarcinoma NT2/D1 cells, are indispensable for normal gonadal development in *Caenorhabditis elegans* (44) and regulate the fragmentation of spermatogonial cysts in mice (45), are not expressed in CIS cells (36). It therefore seems more likely that BLIMP1/PRMT5 controls repression of *HOX* genes and somatic differentiation in CIS cells. Taken together, the emerging picture of the epigenetic profile of CIS cells is an open and permissive chromatin structure that resembles the profile found in fetal germ cells.

Malignant transformation of the neoplastic CIS cells results in either a seminoma or a non-seminoma, and each tumor type develops a distinct epigenetic profile. In general, the non-seminoma, embryonal carcinoma, gains high levels of 5mC (and concurrent upregulation of DNMTs) but retains permissive histone modifications, whereas the seminoma maintains low levels of 5mC but loses the permissive histone modifications (36, 37, 46). In accordance with this, most seminomas also have high levels of H4/H2AR3me2, whereas low levels are found in non-seminomas (39). In summary, adult totipotent CIS cells retain a fetal germ-cell-like epigenetic profile that, after malignant transformation, evolves into distinct signatures reflecting the tumor types.

Epigenetic modifiers

Epigenetic regulation is a dynamic process where modifications like methyl-groups are not only added to DNA and histones but are also actively removed by demethylases with different specificity. Three classes of histone demethylases have been discovered [reviewed by Klose et al. (47)]. The first class is represented by PADI4 (Peptidylarginine deiminase 4), which converts methylarginine to citrulline and thus cannot strictly be categorized as a demethylase. The founding member of the second class of enzymes is LSD1 (Lysine specific demethylase 1), which removes methyl groups from mono- and dimethylated H3K4 and H3K9 in an oxidative manner and with flavin as a cofactor. The third and largest group of demethylases is constituted by jumonji C (JmjC) domain-containing enzymes, which specifically demethylate mono-, di-, and tri-methylated histone lysines in a reaction dependent on iron and α-ketoglutarate. Thirty proteins with an evolutionarily conserved JmjC domain have been identified. So far, 18 jumonji demethylases have documented demethylase activity toward H3K4, H3K9, H3K27, H3K36 or H4K20 [reviewed by Franci et al. (48)]. JmjC proteins have been shown in mice to be necessary for neural tube formation (49), normal heart development (50) and proper testes formation and function (51–53), and their deregulation has been found in numerous tumors, including colon, lung, hematological, pancreatic and breast cancer (54).

Jumonji and testicular development

Fetal and postnatal testicular development is an intricate process where precise epigenetic regulation and derived gene expression cues are pivotal for normal testes function in adulthood (see above). Several studies of JmjC proteins have revealed roles related to germ-cell development, sex determination, fertility and metabolism (51–53, 55).

JMJD1A is necessary for normal spermatogenesis in mice

In 2007, Okada et al. reported that knockout (KO) of *Jmjd1A* (also known as *Kdm3a*, *Jhmd2a* or testis-specific gene A) in C57BL/6 mice led to infertility and small testes (51). Infertility was caused by the failure of spermatids to elongate due to impaired chromatin condensation and could be related

to a decreased expression of two testis-specific basic proteins; transition nuclear protein 1 (TNP1) and protamine 1 (PRM1) in round spermatids of KO mice. In wild-type mice, both JMJD1A protein and transcript were found to be moderately expressed in spermatocytes and highly expressed in round spermatids, and chromatin immunoprecipitation (ChIP) analysis revealed that JMJD1A is recruited to the core promoter regions of *Tnp1* and *Prm1*. Genome-wide H3K9 methylation levels were unaltered, but higher levels were found at the promoters of *Tnp1* and *Prm1* in KO vs. wild type. Probably as a consequence of impaired chromatin condensation, elongating spermatids showed abnormal sperm head morphology and resulted in most spermatozoa being immotile. Surprisingly, there were no differences in hormone levels between wild-type and KO mice.

Liu et al. (52) created another *Jmjd1a* KO mouse model, also in a C57BL/6 background, that in concordance with the previous findings by Okada et al. (51) exhibited small testes, blocked spermatid elongation and infertility. However, in contrast to Okada et al., Liu et al. found that JMJD1A deficiency caused a general increase in H3K9me2 and H3K9me1 levels when assayed by Western blot analysis of whole testes lysates. Moreover, extensive germ-cell apoptosis was reported in spermatocytes and in round and elongating spermatids and resulted in a 1000-fold reduction in the amount of spermatozoa found in the epididymis. Transcriptional regulation of *Tnp1* and *Prm1* is mainly facilitated by a cAMP-response element modulator (CREM) and coactivator ACT (56–58). Liu et al. found decreased levels of *Act* mRNA but not *Crem* mRNA in *Jmjd1a*-deficient mice. However, there was a decreased recruitment of CREM to the promoters of *Tnp1* and *Odf1*. *Jmjd1a* deficiency caused increased H3K9me2/me1 levels at both genes and a decreased expression of CREM and ACT target genes, including *Tnp1*, *Tnp2*, *Prm1*, *Prm2*, *Odf1* and *Gsg3*. These results demonstrate that JMJD1A, by regulating repressive histone marks H3K9me2 and H3K9me1, is necessary for expression of *Act*, recruitment of CREM and expression of their target genes.

JMJD1A is necessary for expression of Sry and Sox9

In 2013, Kuroki et al. showed that KO of the same jumonji gene (*Jmjd1a)* in a C57BL/6 mice strain with a Y-chromosome from CBA embryonal stem cells frequently led to partial or complete sex reversal in XY animals with development of ovotestes or ovaries (53). Interestingly, this effect was greatly dependent on the genetic origin of the Y-chromosome. A CBA origin was the most sensitive, where 88% of XY JMJD1A-deficient mice displayed abnormal sex differentiation compared to 14% in mice with the Y-chromosome coming from C57BL/6 embryonic stem cells. All XY *Jmjd1a*$^{+/+}$ and XY *Jmjd1a*$^{+/\Delta}$ mice developed two testes. In concordance with previous studies (see above), spermiogenesis defects were observed in mice of both backgrounds. JMJD1A was dispensable for both female sex differentiation and female fertility. A very important finding was that JMJD1A deficiency reduced the expression of the protestis mammalian Y-chromosome sex-determining gene *Sry* to approximately 30% in the gonads of both mice strains at embryonic day 11.5 (E11.5). The group also demonstrated with a ChIP analysis that JMJD1A directly binds to regulatory regions within the *Sry* locus in wild type gonadal somatic cells at E11.5 and that *Jmjd1a* deficiency led to a significant increase in H3K9me2 levels within the *Sry* locus. Concordantly the levels of the activating histone mark H3K4me2 decreased in KO mice compared to wild types. It is therefore likely that JMJD1A directly activates the expression of *Sry* by removing suppressive H3K9me2 marks and thereby allowing the methylation of H3K4, which acts as an activator of transcription. If *Sry* is not expressed, the default path will lead to ovarian development. However, minor or local disturbances in the process of sex determination might lead to dysgenetic male gonadal development and could consequently be involved in lack of maturation and neoplastic transformation of germ cells. In principle, the degree of JMJD1A regulation of Sry locus could determine the degree of dysgenetic development.

The expression of the Sertoli cell marker *Sox9* was also significantly reduced at E11.5 in KO mice, while at the same time the number of gonadal somatic cells were unaltered between controls and mutants. However, H3K9me2 levels on *Sox9* were unchanged by JMJD1A deficiency, indicating an indirect mode of regulation.

JMJD1A facilitates transcription activation through the androgen receptor

JMJD1A contains an ^{885}LXXLL^{889} sequence, which is a signature motif involved in nuclear hormone-receptor interaction (59), and the protein has been found to bind to the androgen receptor (AR) in a ligand-dependent

manner *in vitro*. Furthermore, *in vivo* studies with LNCaP cells (androgen-sensitive human prostate adenocarcinoma cells) demonstrated that treatment with synthetic androgen R1881 increased the recruitment of JMJD1A to enhancers of the known AR target genes *PSA* (KLK3; prostate specific antigen) and *NKX3-1* (NK3 homeobox 1) concomitant with a decrease in the level of H3K9me2. Knockdown of *Jmjd1a* led to a significant reduction in hormone-induced transcription (60). Together this indicates that JMJD1A functions as a co-activator of transcription by the androgen receptor. Clinically a link between obesity, testosterone levels and male subfertility is well known, and it is therefore relevant to note that JMJD1A deficiency has also been reported to cause obesity in male mice (55). This was manifested as increased body fat deposition and higher serum lipid content and was possibly caused by an altered expression of metabolic genes, which caused impaired β-oxidation and glycerol release in skeletal muscle. In line with JMJD1A, JMJD2A, B and C (KDM4A, B and C) have also been reported to enhance AR stability and regulate its transcription (61, 62).

Taken together, JMJD1A seems to be specifically involved in crucial steps of proper gonadal development (sex determination) and in maintaining function of the developed testis (DNA condensation and AR signalling).

Other jumonji C domain-containing proteins and testis function

Knockout of *Jmjd1c* in mice (63) resulted in an age-dependent infertility caused by an age-dependent increase in apoptosis of germ cells. JMJD1C was found primarily in undifferentiated spermatogonia, and KO therefore probably perturbed the supply of undifferentiated spermatogonia. Global H3K9 methylation was unaffected in the KO mice, and it has been questioned whether JMJD1C actually contain H3K9 demethylase activity (64).

JMJD1C has also been reported to regulate the expression of CYP17A1, also known as 17α-hydroxylase, via SF-1-mediated transcription (65). CYP17A1 is a key enzyme in the synthesis of androgens and converts pregnenolone and progesterone to DHEA and androstenedione, respectively.

JMJD3 (KDM6B) has been reported to be involved in regulating the fragmentation of spermatogonial cysts, and down-regulation of *Jmjd3* in spermatogonial stem cells have been reported to increase the number of undifferentiated spermatogonia without affecting their differentiation (45). In fetal germ cells and in adult CIS cells, JMJD3 is not expressed (Figure 2) and could play a role in securing the high proliferation rates of CIS cells (36).

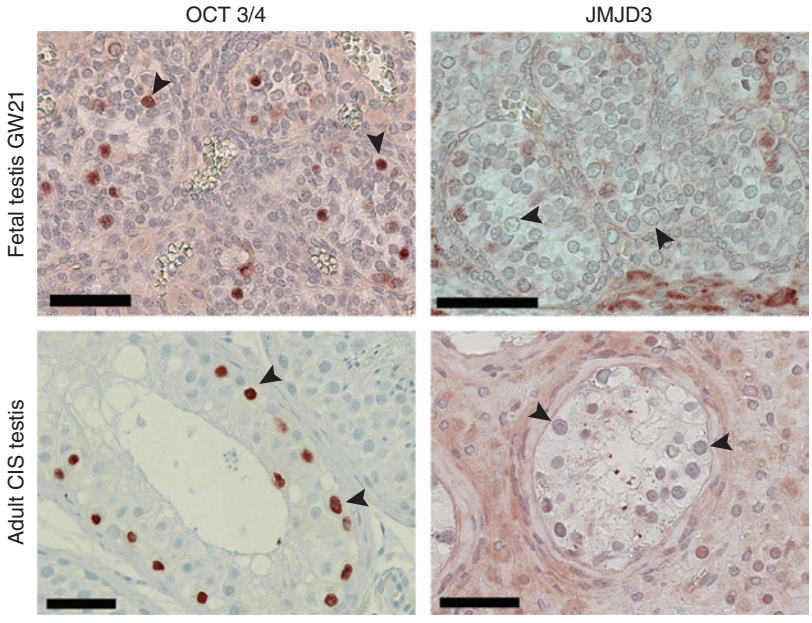

Figure 2: Immunohistochemical staining of gestational-week-21 gonads and adult testis containing CIS, with antibodies directed against OCT 3/4 (POU5F1) and JMJD3.
Arrows indicate fetal germ cells and CIS cells, respectively. JMJD3 is not found expressed in fetal gonads or CIS cells, whereas OCT 3/4 is a classical marker of both. Images are amended from Almstrup et al. (36), where staining patterns in other tissues are also reported. The scale bar represents 50 microns.

In summary, several jumonji C domain-containing proteins have been shown to play important roles at multiple levels of testicular development. JMJD1A seems particularly important for testis function, but it is unknown to what extent these enzymes can compensate each other, especially in humans, where very little is known about the functional roles of jumonji C domain-containing proteins.

Expert opinion

Multiple lines of evidence suggest a close relationship between sex determination, testicular dysgenesis and development of testicular cancer. Germ cells undergo a tightly regulated epigenetic program as they develop and consequently position epigenetic modifiers as key players. Jumonji C domain-containing proteins seem to be implicated in many of the processes that are necessary for both proper fetal testicular development and maintenance of testicular function in adult life. However, the importance of JmjC proteins in human germ-cell development remains to be established.

Outlook

It is a puzzle how histone demethylases can be specific for certain cell types, certain genomic regions and different time points during development. How can JMJD1A be recruited specifically to promoters of *Sry*, *Tnp1* and *Prm1* but not to other genes? JMJD1A has no defined DNA-binding motif, and there is no obvious consensus DNA sequence in the *Tnp1* and *Prm1* promoters. Can other proteins be involved? Moreover, it remains unknown to what extent the family of histone demethylases can compensate each other in the process of human germ-cell development. To describe this complex spatio-temporal relationship, massive genome-wide mapping of epigenetic marks along with its modifiers on a single cell basis is needed.

Highlights

- Testicular germ cell tumors (TGCTs) are the most common cancers among young men.
- Evidence indicates a close similarity in the aetiology of testicular dysgenesis and TGCT.
- Testicular germ cell cancer originates from a totipotent carcinoma *in situ* (CIS) cell.
- CIS cells resemble fetal germ cells and retain an open and permissive chromatin structure in the adult testis.
- Upon malignant transformation, CIS cells develop into two epigenetically distinct tumor types: seminomas and non-seminomas.
- Jumonji C domain-containing (JmjC) proteins specifically demethylate histone lysines.
- Dysregulation of JmjC proteins in mice and human cancer cell lines has recently been reported to cause various degrees of sex reversal, infertility and smaller testes and to influence androgen action.
- We speculate that the regulation of JmjC proteins may be central in the aetiology and pathogenesis of testicular dysgenesis, including testicular cancer.

List of abbreviations

5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
AR	androgen receptor
ChIP	chromatin immunoprecipitation
CIS	carcinoma *in situ*
TDS	testicular dysgenesis syndrome
TGCT	testicular germ cell tumor

References

1. Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. J Urol 2003; 170: 5–11.
2. Chia V, Quraishi S, Devesa S, Purdue M, Cook M, McGlynn K. International trends in the incidence of testicular cancer, 1973–2002. Cancer Epidemiol Biomarkers Prev 2010; 19: 1151–9.
3. Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A, Bray F. International testicular cancer incidence trends: generational transitions in 38 countries 1900–1990. Cancer Causes Control 2015; 26: 151–8.
4. Ulbright T, Amin M, Young R. Atlas of tumor pathology. Tumors of the testis, adnexa, spermatic cord, and scrotum. Washington DC: Armed Forces Institute of Pathology, 1999.
5. Rajpert-De Meyts E, Bartkova J, Samson M, Hoei-Hansen C, Frydelund-Larsen L, Bartek J, Skakkebaek NE. The emerging phenotype of the testicular carcinoma in situ germ cell. APMIS 2003; 111: 267–78; discussion 78–9.
6. Almstrup K, Hoei-Hansen C, Wirkner U, Blake J, Schwager C, Ansorge W, Nielsen JE, Skakkebaek NE, Rajpert-De Meyts E, Leffers H. Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. Cancer Res 2004; 64: 4736–43.
7. Almstrup K, Leffers H, Lothe R, Skakkebaek N, Sonne S, Nielsen J, Rajpert-De Meyts E, Skotheim RI. Improved gene expression signature of testicular carcinoma in situ. Int J Androl 2007; 30: 292–302; discussion 3.

8. Sonne S, Almstrup K, Dalgaard M, Juncker A, Edsgard D, Ruban L, Harrison NJ, Schwager C, Abdollahi A, Huber PE, Brunak S, Gjerdrum LM, Moore HD, Andrews PW, Skakkebaek NE, Rajpert-De Meyts E, Leffers H. Analysis of gene expression profiles of microdissected cell populations indicates that testicular carcinoma in situ is an arrested gonocyte. Cancer Res 2009; 69: 5241–50.
9. Novotny GW, Belling KC, Bramsen JB, Nielsen JE, Bork-Jensen J, Almstrup K, Sonne SB, Kjems J, Rajpert-De Meyts E, Leffers H. MicroRNA expression profiling of carcinoma in situ cells of the testis. Endocr Relat Cancer 2012; 19: 365–79.
10. Hoei-Hansen C, Almstrup K, Nielsen J, Brask Sonne S, Graem N, Skakkebaek N, Leffers H, Rajpert-De Meyts E. Stem cell pluripotency factor NANOG is expressed in human fetal gonocytes, testicular carcinoma in situ and germ cell tumours. Histopathology 2005; 47: 48–56.
11. Hoei-Hansen C, Nielsen J, Almstrup K, Sonne S, Graem N, Skakkebaek NE, Leffers H, Rajpert-De Meyts E. Transcription factor AP-2gamma is a developmentally regulated marker of testicular carcinoma in situ and germ cell tumors. Clin Cancer Res 2004; 10: 8521–30.
12. Rajpert-De Meyts E, Hanstein R, Jorgensen N, Graem N, Vogt PH, Skakkebaek NE. Developmental expression of POU5F1 (OCT-3/4) in normal and dysgenetic human gonads. Hum Reprod 2004; 19: 1338–44.
13. Looijenga L, Stoop H, de Leeuw H, de Gouveia Brazao C, Gillis A, van Roozendaal K, van Zoelen EJ, Weber RF, Wolffenbuttel KP, van Dekken H, Honecker F, Bokemeyer C, Perlman EJ, Schneider DT, Kononen J, Sauter G, Oosterhuis JW. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 2003; 63: 2244–50.
14. Rajpert-De Meyts E, Skakkebaek NE. Expression of the c-kit protein product in carcinoma-in-situ and invasive testicular germ cell tumours. Int J Androl 1994; 17: 85–92.
15. Oosterhuis J, Castedo S, de Jong B, Cornelisse C, Dam A, Sleijfer D, Schraffordt Koops H. Ploidy of primary germ cell tumors of the testis. Pathogenetic and clinical relevance. Lab Invest 1989; 60: 14–21.
16. Ottesen A, Skakkebaek N, Lundsteen C, Leffers H, Larsen J, Rajpert-De Meyts E. High-resolution comparative genomic hybridization detects extra chromosome arm 12p material in most cases of carcinoma in situ adjacent to overt germ cell tumors, but not before the invasive tumor development. Genes Chromosomes Cancer 2003; 38: 117–25.
17. Rosenberg C, Van Gurp R, Geelen E, Oosterhuis J, Looijenga L. Overrepresentation of the short arm of chromosome 12 is related to invasive growth of human testicular seminomas and nonseminomas. Oncogene 2000; 19: 5858–62.
18. Atkin N, Baker M. Specific chromosome change, i(12p), in testicular tumours? Lancet 1982; 2: 1349.
19. Rajpert-De Meyts E. Developmental model for the pathogenesis of testicular carcinoma in situ: genetic and environmental aspects. Hum Reprod Update 2006; 12: 303–23.
20. Kristensen DM, Sonne SB, Ottesen AM, Perrett RM, Nielsen JE, Almstrup K, Skakkebaek NE, Leffers H, Rajpert-De Meyts E. Origin of pluripotent germ cell tumours: the role of microenvironment during embryonic development. Mol Cell Endocrinol 2008; 288: 111–8.
21. Jorgensen A, Nielsen JE, Almstrup K, Toft BG, Petersen BL, Rajpert-De Meyts E. Dysregulation of the mitosis-meiosis switch in testicular carcinoma in situ. J Pathol 2013; 229: 588–98.
22. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 2001; 16: 972–8.
23. Hemminki K, Li X. Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. Br J Cancer 2004; 90: 1765–70.
24. Kanetsky PA, Mitra N, Vardhanabhuti S, Vaughn DJ, Li M, Ciosek SL, Letrero R, D'Andrea K, Vaddi M, Doody DR, Weaver J, Chen C, Starr JR, Håkonarson H, Rader DJ, Godwin AK, Reilly MP, Schwartz SM, Nathanson KL. A second independent locus within DMRT1 is associated with testicular germ cell tumor susceptibility. Hum Mol Genet 2011; 20: 3109–17.
25. Chung CC, Kanetsky PA, Wang Z, Hildebrandt MA, Koster R, Skotheim RI, Kratz CP, Turnbull C, Cortessis VK, Bakken AC, Bishop DT, Cook MB, Erickson RL, Fosså SD, Jacobs KB, Korde LA, Kraggerud SM, Lothe RA, Loud JT, Rahman N, Skinner EC, Thomas DC, Wu X, Yeager M, Schumacher FR, Greene MH, Schwartz SM, McGlynn KA, Chanock SJ, Nathanson KL. Meta-analysis identifies four new loci associated with testicular germ cell tumor. Nat Genet 2013; 45: 680–5.
26. Kanetsky PA, Mitra N, Vardhanabhuti S, Li M, Vaughn DJ, Letrero R, Ciosek SL, Doody DR, Smith LM, Weaver J, Albano A, Chen C, Starr JR, Rader DJ, Godwin AK, Reilly MP, Hakonarson H, Schwartz SM, Nathanson KL. Common variation in KITLG and at 5q31.3 predisposes to testicular germ cell cancer. Nat Genet 2009; 41: 811–5.
27. Ruark E, Seal S, McDonald H, Zhang F, Elliot A, Lau K, Perdeaux E, Rapley E, Eeles R, Peto J, Kote-Jarai Z, Muir K, Nsengimana J, Shipley J, UK Testicular Cancer Collaboration (UKTCC), Bishop DT, Stratton MR, Easton DF, Huddart RA, Rahman N, Turnbull C. Identification of nine new susceptibility loci for testicular cancer, including variants near DAZL and PRDM14. Nat Genet 2013; 45: 686–9.
28. Turnbull C, Rapley EA, Seal S, Pernet D, Renwick A, Hughes D, Ricketts M, Linger R, Nsengimana J, Deloukas P, Huddart RA, Bishop DT, Easton DF, Stratton MR, Rahman N, UK Testicular Cancer Collaboration. Variants near DMRT1, TERT and ATF7IP are associated with testicular germ cell cancer. Nat Genet 2010; 42: 604–7.
29. Rapley EA, Turnbull C, Al Olama AA, Dermitzakis ET, Linger R, Huddart RA, Renwick A, Hughes D, Hines S, Seal S, Morrison J, Nsengimana J, Deloukas P; UK Testicular Cancer Collaboration, Rahman N, Bishop DT, Easton DF, Stratton MR. A genome-wide association study of testicular germ cell tumor. Nat Genet 2009; 41: 807–10.
30. Koster R, Mitra N, D'Andrea K, Vardhanabhuti S, Chung CC, Wang Z, Loren Erickson R, Vaughn DJ, Litchfield K, Rahman N, Greene MH, McGlynn KA, Turnbull C, Chanock SJ, Nathanson KL, Kanetsky PA. Pathway-based analysis of GWAs data identifies association of sex determination genes with susceptibility to testicular germ cell tumors. Hum Mol Genet 2014; 23: 6061–8.
31. Dalgaard MD, Weinhold N, Edsgard D, Silver JD, Pers TH, Nielsen JE, Jørgensen N, Juul A, Gerds TA, Giwercman A, Giwercman YL, Cohn-Cedermark G,Virtanen HE, Toppari J, Daugaard G, Jensen TS, Brunak S, Rajpert-De Meyts E, Skakkebæk NE, Leffers H, Gupta R. A genome-wide association study of men with symptoms of testicular dysgenesis syndrome and its network biology interpretation. J Med Genet 2012; 49: 58–65.

32. Hoei-Hansen CE, Holm M, Rajpert-De Meyts E, Skakkebaek NE. Histological evidence of testicular dysgenesis in contralateral biopsies from 218 patients with testicular germ cell cancer. J Pathol 2003; 200: 370–4.
33. Skakkebaek NE, Holm M, Hoei-Hansen C, Jorgensen N, Rajpert-De Meyts E. Association between testicular dysgenesis syndrome (TDS) and testicular neoplasia: evidence from 20 adult patients with signs of maldevelopment of the testis. APMIS 2003; 111: 1–11.
34. Olesen IA, Hoei-Hansen CE, Skakkebaek NE, Petersen JH, Meyts ERD, Jorgensen N. Testicular carcinoma in situ in subfertile Danish men. Int J Androl 2007; 30: 406–11.
35. Peng X, Zeng X, Peng S, Deng D, Zhang J. The association risk of male subfertility and testicular cancer: a systematic review. PLoS One 2009; 4: e5591.
36. Almstrup K, Nielsen JE, Mlynarska O, Jansen MT, Jorgensen A, Skakkebaek NE, Rajpert-De Meyts E. Carcinoma in situ testis displays permissive chromatin modifications similar to immature foetal germ cells. Br J Cancer 2010; 103: 1269–76.
37. Netto G, Nakai Y, Nakayama M, Jadallah S, Toubaji A, Nonomura N, Albadine R, Hicks JL, Epstein JI, Yegnasubramanian S, Nelson WG, De Marzo AM. Global DNA hypomethylation in intratubular germ cell neoplasia and seminoma, but not in nonseminomatous male germ cell tumors. Mod Pathol 2008; 21: 1337–44.
38. Kristensen DG, Nielsen JE, Jorgensen A, Skakkebaek NE, Rajpert-De Meyts E, Almstrup K. Evidence that active demethylation mechanisms maintain the genome of carcinoma in situ cells hypomethylated in the adult testis. Br J Cancer 2014; 110: 668–78.
39. Eckert D, Biermann K, Nettersheim D, Gillis A, Steger K, Jäck H, Müller AM, Looijenga LH, Schorle H. Expression of BLIMP1/PRMT5 and concurrent histone H2A/H4 arginine 3 dimethylation in fetal germ cells, CIS/IGCNU and germ cell tumors. BMC Dev Biol 2008; 8: 106.
40. Ohinata Y, Payer B, O'Carroll D, Ancelin K, Ono Y, Sano M, Barton SC, Obukhanych T, Nussenzweig M, Tarakhovsky A, Saitou M, Surani MA. Blimp1 is a critical determinant of the germ cell lineage in mice. Nature 2005; 436:207–13.
41. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 2006; 125: 301–13.
42. Kirmizis A, Bartley SM, Kuzmichev A, Margueron R, Reinberg D, Green R, Farnham PJ. Silencing of human polycomb target genes is associated with methylation of histone H3 Lys 27. Genes Dev 2004; 18: 1592–605.
43. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 2002; 298: 1039–43.
44. Agger K, Cloos P, Christensen J, Pasini D, Rose S, Rappsilber J, Issaeva I, Canaani E, Salcini AE, Helin K. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 2007; 449: 731–4.
45. Iwamori N, Iwamori T, Matzuk MM. H3K27 demethylase, JMJD3, regulates fragmentation of spermatogonial cysts. PLoS One 2013; 8: e72689.
46. Almstrup K, Hoei-Hansen C, Nielsen J, Wirkner U, Ansorge W, Skakkebaek N, Rajpert-De Meyts E, Leffers H. Genome-wide gene expression profiling of testicular carcinoma in situ progression into overt tumours. Br J Cancer 2005; 92: 1934–41.
47. Klose RJ, Kallin EM, Zhang Y. JmjC-domain-containing proteins and histone demethylation. Nature Rev Gen 2006; 7: 715–27.
48. Franci G, Ciotta A, Altucci L. The Jumonji family: past, present and future of histone demethylases in cancer. Biomol Concepts 2014; 5: 209–24.
49. Takeuchi T, Yamazaki Y, Katoh-Fukui Y, Tsuchiya R, Kondo S, Motoyama J, Higashinakagawa T. Gene trap capture of a novel mouse gene, jumonji, required for neural tube formation. Genes Dev 1995; 9: 1211–22.
50. Lee Y, Song AJ, Baker R, Micales B, Conway SJ, Lyons GE. Jumonji, a nuclear protein that is necessary for normal heart development. Circ Res 2000; 86: 932–8.
51. Okada Y, Scott G, Ray MK, Mishina Y, Zhang Y. Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis. Nature 2007; 450: 119–23.
52. Liu Z, Zhou S, Liao L, Chen X, Meistrich M, Xu J. Jmjd1a demethylase-regulated histone modification is essential for cAMP-response element modulator-regulated gene expression and spermatogenesis. J Biol Chem 2010; 285: 2758–70.
53. Kuroki S, Matoba S, Akiyoshi M, Matsumura Y, Miyachi H, Mise N, Abe K, Ogura A, Wilhelm D, Koopman P, Nozaki M, Kanai Y, Shinkai Y, Tachibana M. Epigenetic regulation of mouse sex determination by the histone demethylase Jmjd1a. Science 2013; 341: 1106–9.
54. van Haaften G, Dalgliesh GL, Davies H, Chen L, Bignell G, Greenman C, Edkins S, Hardy C, O'Meara S, Teague J, Butler A, Hinton J, Latimer C, Andrews J, Barthorpe S, Beare D, Buck G, Campbell PJ, Cole J, Forbes S, Jia M, Jones D, Kok CY, Leroy C, Lin ML, McBride DJ, Maddison M, Maquire S, McLay K, Menzies A, Mironenko T, Mulderrig L, Mudie L, Pleasance E, Shepherd R, Smith R, Stebbings L, Stephens P, Tang G, Tarpey PS, Turner R, Turrell K, Varian J, West S, Widaa S, Wray P, Collins VP, Ichimura K, Law S, Wong J, Yuen ST, Leung SY, Tonon G, DePinho RA, Tai YT, Anderson KC, Kahnoski RJ, Massie A, Khoo SK, Teh BT, Stratton MR, Futreal PA. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet 2009; 41: 521–3.
55. Tateishi K, Okada Y, Kallin EM, Zhang Y. Role of Jhdm2a in regulating metabolic gene expression and obesity resistance. Nature 2009; 458: 757–61.
56. Mali P, Kaipia A, Kangasniemi M, Toppari J, Sandberg M, Hecht NB, Parvinen M. Stage-specific expression of nucleoprotein mRNAs during rat and mouse spermiogenesis. Reprod Fertil Dev 1989; 1: 369–82.
57. Nantel F, Monaco L, Foulkes NS, Masquilier D, LeMeur M, Henriksen K, Dierich A, Parvinen M, Sassone-Corsi P. Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. Nature 1996; 380: 159–62.
58. Fimia GM, De Cesare D, Sassone-Corsi P. CBP-independent activation of CREM and CREB by the LIM-only protein ACT. Nature 1999; 398: 165–9.
59. Heery DM, Kalkhoven E, Hoare S, Parker MG. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 1997; 387: 733–6.
60. Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y. JHDM2A, a JmjC-containing H3K9

demethylase, facilitates transcription activation by androgen receptor. Cell 2006; 125: 483–95.
61. Coffey K, Rogerson L, Ryan-Munden C, Alkharaif D, Stockley J, Heer R, Sahadevan K, O'Neill D, Jones D, Darby S, Staller P, Mantilla A, Gaughan L, Robson CN. The lysine demethylase, KDM4B, is a key molecule in androgen receptor signalling and turnover. Nucleic Acids Res 2013; 41: 4433–46.
62. Shin S, Janknecht R. Activation of androgen receptor by histone demethylases JMJD2A and JMJD2D. Biochem Biophys Res Comm 2007; 359: 742–6.
63. Kuroki S, Akiyoshi M, Tokura M, Miyachi H, Nakai Y, Kimura H, Shinkai Y, Tachibana M. JMJD1C, a JmjC domain-containing protein, is required for long-term maintenance of male germ cells in mice. Biol Reprod 2013; 89: 93.
64. Brauchle M, Yao Z, Arora R, Thigale S, Clay I, Inverardi B, Fletcher J, Taslimi P, Acker MG, Gerrits B, Voshol J, Bauer A, Schübeler D, Bouwmeester T, Ruffner H. Protein complex interactor analysis and differential activity of KDM3 subfamily members towards H3K9 methylation. PLoS One 2013; 8: e60549.
65. Kim SM, Kim JY, Choe NW, Cho IH, Kim JR, Kim DW, Seol JE, Lee SE, Kook H, Nam KI, Kook H, Bhak YY, Seo SB. Regulation of mouse steroidogenesis by WHISTLE and JMJD1C through histone methylation balance. Nucleic Acids Res 2010; 38: 6389–403.