

Oct-4: More than a Pluripotent Marker?

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Abstract

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Oct-4/ Pou5f1/ Oct-3, a POU domain family protein acts as a crucial transcription factor during embryonic development. It helps in maintenance of self renewal as well as pluripotential state of embryonic stem (ES) cells. Its expression starts right from 2 cell stage especially prior to 8 cell stage till the blastocyst stage where it is strongly expressed in inner cell mass (ICM). Thereafter, it is located predominantly in primordial germ cells (PGCs) till the birth. It targets particularly those genes which bear an octameric motif ATGCAAAT in promoter or enhancer region. Most of the target genes of Oct-4 are expressed in undifferentiated ES cells and knockdown of Oct-4 results in ES cell differentiation as a result of down regulation of targets of Oct-4 which are expressed in ES cells. Since, Oct-4 is crucial for embryo survival its expression needs tight regulation. Oct-4 is carefully regulated epigenetically as well as by several other factors. DNA methylation and histone modification play an important role in expression of Oct-4 while proximal promoter, enhancer and distal enhancer are the crucial regulatory elements present on Oct-4 upstream region. There is increasing evidence that Oct-4 is expressed in adult stem cells and these stem cells can get converted to cancer stem cells. Since, it is expressed in germ cells, immunohistochemical localization of Oct-4 and thereby its role as a marker for germ cell tumor detection is increasing. Thus, role of Oct-4 is not only restricted as a marker for undifferentiated cells; it is also proving to be crucial factor which targets several genes involved in ES cell survival as well as help in establishing germ cell origin of metastatic tumors.

Keywords: Oct-4/Pou5f1/Oct-3, POU, Embryonic Stem Cell, ICM

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Introduction

Oct-4 is a class V Pit-1, Oct-4, Unc-86 (POU) domain family of octamer binding transcription factor (TF) that possesses POU specific (POUs) and POU-homeodomain (POU_{HD}) (1, 2). The members of POU family of TFs activate the transcription of genes bearing cis-acting elements, which contains an octamer motif, ATGCAAAT (3). During embryonic development Oct-4 binds to this octamer site found in promoter or enhancer regions of many genes (4).

Oct-4: transcription factor

Oct-4 gene maps to chromosome number 6 in humans and chromosome 17 in mouse. It is separated into 5 exons (5, 6). Oct-4 upstream region consists of a proximal promoter upstream of transcription start site, proximal and distal enhancers. All three regions are important regulatory elements during embryonic development (7). Oct-4 encodes mRNA transcript of 1.5 Kb and consists of 352 amino acids (8, 9).

Since, Oct-4 is a TF it regulates target gene function. Pan et al (10) have identified a nuclear localization signal motif (NLS) motif RKRKR and found that mutation

of this motif results in dominant negative mutant capable of inhibiting Oct-4 activity. These authors further showed that NLS mutant co expressed with wild type Oct-4 causes dimerization with wild type Oct-4 thereby trapping it in cytosol and preventing it from transactivating target. In the nucleus, Oct-4 distribution differs in transcriptionally active and inactive mouse oocytes. Parfenov and group (11) have deduced its relation to RNA polymerase II and splicing factors. They further showed that Oct-4 is localized in perichromatin fibrils (PFs), interchromatin granule clusters (IGCs) and in dense fibrillar component (DFC) of nucleolus at transcriptionally active stage of oocyte nucleus. Oct-4 present in PFs and IGCs co localizes with Pol II and SC35 (splicing factor) at transcriptionally active stage. Oct-4 accumulates in nucleolus like bodies (NCBs), cajal bodies (CBs), perichromatin granules (PGs) at inert stage of oocyte. The co localization of Oct-4, Pol II and SC35 with coilin containing structures such as NCBs, CBs at the inert stage (antral follicles) suggests that latter may represent storage sites for transcription or splicing machinery during decline of transcription.

Expression of Oct-4

Octamer binding proteins confer transcriptional activity in early mouse embryogenesis was demonstrated for the first time by Scholer and his group (12). They analyzed the activity of octamer motif in two ES cell lines (F9 and D3) expressing both Oct-4 and Oct-5. After differentiation, enhancer activity of oligomerized octamer motif and octamer binding proteins decrease indicating Oct-4 and Oct-5 are active in early mouse development. However, subsequently it has been shown that Oct-4 is expressed throughout preimplantation period (8, 9, 13). Oct-4 mRNA and protein are present in unfertilized oocytes and protein is localized in pronuclei following fertilization as well as in two cell embryos (6, 13, 14). Zygotic Oct-4 expression is activated prior to 8-cell stage (6, 14). It is detected at 8-16 cell morula stage, which increases in early blastocyst and then declines in late blastocyst. Expression of Oct-4 mRNA and protein is abundant and uniform in all cells of embryo through morula stage. But, when these cells differentiate to inner cell mass (ICM) and trophoctoderm (TE) Oct-4 is maintained only in ICM and down regulated in TE (8, 13, 15). Thereafter, expression is localized in primitive ectoderm (7, 13).

At 7.5 dpc, expression of Oct-4 during embryogenesis is restricted only to primordial germ cells (PGCs). Using Oct-4-GFP construct, the expression of Oct-4 has been studied in transgenic mice through all stages of germ cell development. Oct-4 is expressed in both male and female germ cells at all developmental stages. Oct-4 mRNA as well as protein expression is present in the nuclei of proliferating PGCs present in undifferentiated genital ridges at 11.5 dpc and in developing ovaries and testes at 12.5 dpc and 13.5 dpc (7, 9, 13, 16). Oct-4 is expressed at comparable levels in PGCs in both sexes until 13.5 dpc, and is down regulated in zygotene/ pachytene female germ cells at 16.5 dpc. In contrast, male germ cells still express Oct-4 at this stage. Oct-4 is expressed at low levels in the cytoplasm of oocytes of new born females at 1dpp. Oct-4 protein expression is up regulated in oocytes within primordial follicles. In growing oocytes in bi/trilaminar follicles at 12dpp and 14dpp and in fully grown oocytes in adult females expression is upregulated (14). It is expressed in mitotically arrested prospermatogonia until birth. It is detected in the entire population of spermatogonia in newborn male mice at 1dpp and 7dpp. These germ cells represent undifferentiated spermatogonia and constitute the stem cell population of germ cells within the testis (16). Yeom and group (7) have used Oct-4-LacZ transgene construct to study expression pattern of Oct-4 during mouse embryonic development. They found down regulation of Oct-4 during gastrulation and then maintained only in germ line lineage. Using Cre/lox P conditional system it has been reported that loss of Oct-4 leads to apoptosis of PGC's rather than differentiation into TE lineage (17).

Differentiation of mouse ES cells after RNAi mediated

silencing of Oct-4 and Nanog leads to differentiation to TE lineage and activation of TE marker genes *Cdx-2*, *Hand-1*, *Pl-1* and extra embryonic endoderm genes *GATA-4*, *GATA-6*, *Laminin B1* (18). Velkey and group (19) showed differentiation of ES cells to TE when Oct-4 RNAi is used. Thus, down regulation of Oct-4 in outer cells leading to TE lineage and maintenance of ICM appears to be one of the crucial events enabling proper preimplantation and embryo development (20). Oct-4 has a conserved function during early brain patterning in mouse and repression accelerates differentiation of neural progenitor cells *in vitro* and *in vivo* (21,22). It also dose dependently regulates specification of ES cells towards a cardiac lineage and early heart development (23). Thus, a critical amount of Oct-4 is required to sustain stem cells self-renewal and up or down regulation induces divergent developmental programme (24). Therefore, expression of Oct-4 in ES cells and its down regulation leading to differentiation of these cells describes its significance in embryo development.

In analogous to mouse Oct-4 expression Oct-4 mRNA and protein expression is detected in human preimplantation embryos as well. Gerrard et al (25) studied the expression of Oct-4-GFP in stably transfected hESC clones, which were found to maintain self-renewal and pluripotency of ES cells. So, Oct-4 maintains totipotency in both humans and mouse species (26). The previous finding also supports these observations that Oct-4 *-/-* homozygous embryos die during implantation due to failure to form ICM. Loss of Oct-4 causes ICM cells to differentiate to TE lineage. Analysis of Oct-4 expression and ploidy in individual human blastomeres has shown differential Oct-4 expression in individual human blastomeres and this appears to direct cells towards ICM/TE irrespective of chromosomal status (27). There are reports about expression of Oct-4 in adult stem cells but not in differentiated cells (28). Thus, Oct-4 is considered to be the important regulator, which is required throughout the embryonic development as well as for embryo survival.

Recently, there have been speculations about the presence of Oct-4 in human amniotic fluid (29) and it has also been found to be present in endometrium of mouse (30) but its role in endometrium is not yet clear. Though, Oct-4 is considered to be expressed only in undifferentiated cells there is a recent report which describes its presence in adult terminally differentiated cells such as peripheral blood mononuclear cells (PBMCs) thereby challenging its role as a pluripotency marker (31). But, it is essential to distinguish between Oct-4A and Oct-4B when interpreting OCT-4 expression in differentiated cells. This is necessary because Oct-4B doesn't share stemness factor characteristics of Oct-4A (32). Overproduction of Oct-4 in mouse ES cell inhibits hematopoietic differentiation in dose dependent manner and leads to prolonged mesoderm commitment *in vitro* (33). In order to understand the role of Oct-4 in murine and human ES cells, many investigators have used Oct-4

knockdown strategy in the past. Oct-4 knockdown led to expression of trophoblast associated transcription factor Cdx-2 as well as expression of endoderm associated genes Gata-6 and α -fetoprotein (Fig 1).

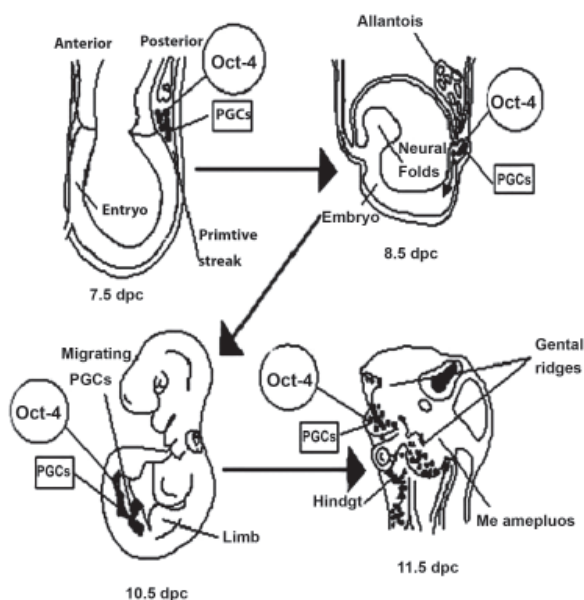


Fig 1: Oct-4 expression during embryogenesis: Oct-4 expression starts from as early as 2 cell stage. It continues to maintain its expression in 8 cells and in morula stage. During blastocyst formation only inner cell mass expresses Oct-4 while trophoectoderm remains Oct-4 negative. At 7.5 dpc only PGCs express Oct-4. Then, Oct-4 is expressed by PGCs right throughout germ cell migration process till it reaches to genital ridges at around 11.5dpc. It continues to express itself in developing testes and ovaries at 12.5 and 13.5 dpc. The expression is down regulated in zygotene/ pachytene stage female germ cells at 16.5dpc while male germ cells still continue to express Oct-4.

Expression of Oct-4 in different species

The role of Oct-4 in maintaining pluripotency of ES cells and undifferentiated state is crucial not only in human and murine ES cells; but also studies have been carried out in porcine, bovine, medaka fish, Rhesus monkeys and chicken ES cells.

To understand further the Oct-4 expression in ICM and trophoectoderm of porcine, bovine and murine blastocyst, a transgene construct GOF18 Δ PE-GFP has been used. Oct-4 is found to be expressed in both ICM and trophoectoderm of porcine and bovine blastocyst but in murine embryos expression is restricted only to ICM. This led to an indication that presence of Oct-4 protein may not be sufficient for selection of undifferentiated cell lines in domestic animals (34). It is further shown that Oct-4 is not restricted to ICM blastocyst of bovine embryos indicating it is not a key regulator of pluripotency, but, temporal and spatial pattern of Oct-4 transcript in bovine oocytes and preimplantation stage embryos led to finding that regulation of Oct-4 transcription is conserved between mammalian species (35).

Hong et al (36) have demonstrated activation of Oct-4 promoter in medaka fish blastula embryos and cells of ES cell lines MES (36). These studies have shown that some transcription factors and cis-acting regulatory elements controlling totipotency specific gene expression appear to be conserved between mammals and fish. Medaka ES cells offer an in vitro system for characterizing expression of totipotency specific genes such as putative Oct-4 homologs (36). Surprisingly, as against the expression pattern of Oct-4 in porcine and bovine blastocyst, Oct-4 expression pattern in rhesus monkeys is consistent with that of mouse pluripotential cells with ICM showing Oct-4 expression and TE showing almost no or very low signal (37). Oct-4 expression has been apparently found to be absent in chicken genome (38). Though, the localization of Oct-4 amongst different animals may differ; temporal and spatial pattern of expression and thereby its regulation is conserved between mammalian species.

Oct-4: target genes

Oct-4 interacts with several transcription factors such as HMG family Sox-2, Fox-D3 and Nanog in order to maintain self-renewal as well as pluripotential state of ES cells. Babaie et al (39) analyzed Oct-4 dependent transcriptional network regulating self-renewal and pluripotency in human ES cells. RNAi mediated suppression of Oct-4 and analysis of resulting transcriptional profiles were used to identify Oct-4 dependent genes in human cells. The altered expression of greater than 1000 genes including targets regulated by OCT-4 either positively (NANOG, SOX-2, REX-1, LEFTB, LEFTA/EBAF DPPA-4, THY-1, and TDGF-1) or negatively (CDX-2, EOMES, BMP-4, TBX-18, Brachyury [T], DKK-1, HLX-1, GATA-6, ID-2, and DLX-5), as well as targets for the OCT4-associated stem cell regulators SOX2 and NANOG has been found to occur. In order to carry out efficient transcription Oct-3/4 display distinct DNA binding specificity from those of other octamer factors (40).

PDGF- α R, hCG α , hCG β and Rex-1

Two different promoters control PDGF- α R, one during undifferentiated state and other during differentiated state. In an undifferentiated state an alternative promoter, contains an octamer motif bound by Oct-4 thereby controlling production of transcripts of 1.5 Kb and 5 Kb size. But, in differentiated (RA mediated differentiation) cells of Tera2 transcripts size is 6.4 Kb (full length receptors) and 3.0 Kb (41).

Human Chorionic gonadotropin (hCG) hormone is important during pregnancy. Oct-4 completely silences the expression of hCG- α and hCG- β subunit by binding to ACAATAATCA motif (42, 43). Similarly, Rex-1 (Zfp-42) a transcription factor expressed in early embryo encodes an acidic Zinc finger protein, which is expressed at higher levels in ES and F9 teratocarcinoma cell lines. Oct-4 activates or represses Rex-1 promoter depending

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on cellular environment. Rex-1 harbors an octamer motif in upstream region of transcription start site (44).

Interferon-Tau

Interferon-tau (IFN- τ) is structurally related to IFN- α , IFN- β and IFN- ω (with approximately 25, 50, and 75% primary sequence identities, respectively) although these IFNs exhibit typical properties, including the ability to induce an antiviral state in cells expressing the appropriate IFN receptor. The pre attachment conceptuses of ruminant species, such as cattle, sheep, and deer, produce IFN-tau in order to prevent the regression of the maternal corpus luteum, an event that would normally occur at the end of the estrous cycle if the animal were not pregnant. Members of the multigene IFN-tau family are expressed weakly in the trophectoderm of cattle beginning when this epithelium differentiates at blastocyst formation. The level of production of IFN-tau per cell increases markedly as the blastocyst enlarges and begins to elongate. Production is down regulated when the trophectoderm forms contacts with the uterine wall and as the process of placentation is initiated. Oct-4 causes silencing of Tau-IFN promoters by quenching Ets-Z transactivation (45).

Oct-4 and Sox-2 complex regulated genes

Oct-4 and HMG family transcription factor Sox-2 interact to either activate or suppress target gene expression. Oct-4 and Sox-2 dimerize onto DNA in different conformational arrangements. DNA enhancer region of target genes particularly Fibroblast growth factor-4 (Fgf-4) and Undifferentiated transcription factor-1 (Utf-1) is responsible for correct spatial arrangement of glue like interaction domains on their surface. Oct-4 requires less Sox-2 to heterodimerize and augment Utf-1 activity than for Fgf-4 (46). In ES cells Utf-1, a transcriptional co activator is expressed. It is also regulated by Oct-4 and Sox-2 but unlike Fgf-4 enhancer, Utf-1 element by its one base difference from canonical octamer-binding sequence selectively recruit complex comprising Oct-4 and Sox-2 preclude binding of transcriptionally inactive complex containing Oct-1/Oct-6 (47). Therefore, Oct-4 maintains proliferative ES cell state via specific binding to variant octamer sequence in regulatory region of UTF-1 locus (48).

Lefty-1, Nanog, Zfp-206 and Fbx-15

Lefty-1, gene, which also harbors ES cell specific enhancer upstream of promoter, is under the control of Oct-4-Sox-2 complex. This enhancer element is not activated by Oct-4/Sox-2 complex in differentiated cells. Along with N-terminal transactivation domain of Oct-4/Sox-2 another factor called Kruppel like factor 4 (Klf-4) is necessary. It cooperates with Oct-4 and Sox-2 to activate Lefty-1 expression. Klf-4 acts as a mediating factor that specifically binds to proximal element of Lefty-1 promoter (49).

Nanog acts as a pluripotential marker in ES cells. In

Nanog proximal promoter, there is a composite sox-oct cis regulatory element essential for Nanog transcription. Oct-4/Sox-2 binds to Nanog promoter in mouse and human ES cells (50). Like Sox-2, β -catenin also up regulates Nanog expression through interaction with Oct-4 in ES cells (51).

ZFP-206, another transcription factor that has a role in maintaining stem cell pluripotency; is a direct downstream target of Oct-4 and Sox-2. Two composite Sox-Oct binding sites have been identified within first intron of Zfp-206. Oct-4 and Sox-2 alone could activate transcription via one of this Sox-Oct element, although the presence of both Oct-4 and Sox-2 gives synergistic effect (52). Apart from Sox-2, Oct-4 interacts with Nanog to control the cascade of pathways that are intricately connected to govern pluripotency, self-renewal, and genome surveillance, cell fate determination. They overlap in their targets and are bound to genes in different configurations (53).

F-box (Fbx-15) is a protein motif of approximately 50 amino acids that functions as a site of protein-protein interaction in which they bind substrates for ubiquitin-mediated proteolysis. F-box containing Fbx-15 is predominantly expressed in undifferentiated ES cells. Fbx-15 has been found to be dispensable for ES cells to maintain normal morphology, proliferation and differentiation (54). Another zinc finger protein, which is a downstream target molecule of Oct-4 in ES cells, is Zfp-57.

Oct-4, Nanog and FoxD3 interaction

Its not only Oct-4/Sox-2 interaction or interaction of Oct-4 with Nanog and FoxD3 which help in maintaining self renewal and pluripotency of ES cells but also Oct-4, Nanog and FoxD3 form a negative feedback loop which is also crucial. Oct-4 maintains Nanog activity by activating its promoter at sub-steady state concentration but repressing its activity at or above steady state level. FoxD3 acts as a positive activator of Nanog overcoming Oct-4 effect. The expression of Oct-4 is activated by FoxD3 and Nanog but is repressed by Oct-4 itself. This is in accordance with the results that state Oct-4, FoxD3 and Nanog are required for ES cells self-renewal and maintain pluripotency (55).

The above discussion suggests that, Oct-4 is not only restricted as a marker of pluripotency but a number of genes, which are involved in chromatin regulation (Smarcc1, Arid1a, 5b, Jarid 1b, Jarid 2), cell cycle control (Ccnf, Cdc-25, Ccnb-2), apoptosis (Aatf, Casp-6, Bin1), DNA repair (Brca, Rad-51, Blm), Nuclear architecture (Pml and Coil) are either regulated positively or negatively by Oct-4 (56).

Somatic cloning: reprogramming of Oct-4 gene

Reprogramming of Oct-4 in somatic cloning

In order to understand Oct-4 expression in somatic clones Boiani et al (57) used Oct-4-GFP transgene construct as marker for which gene reprogramming relates

to developmental potential of somatic clones. Cumulus cell clones initiate Oct-4 expression at correct stage but temporally an incorrect spatial expression in majority of blastocyst. It has been found that the ability of clones to form outgrowth gets reduced and outgrowth shows low or even undetectable levels of Oct-4 RNA/GFP. This could be caused by reprogramming errors or aberrant reactivation or incomplete reactivation of Oct-4 related genes whose expression pattern is related to Oct-4. Oct-4 and ten Oct-4 related genes expression analysis in individual cumulus cell derived cloned blastocysts showed 62% correct expression in all tested genes while in ES cell cloned blastocyst and normal control embryos expression has been found to be normal. The reprogramming has been found to be in the context of changes in methylation status, chromatin structure while transcriptional activity of Oct-4 expression led to extinction of Oct-4 gene expression in EC X fibroblast somatic cell hybrids (57-60). As compared to other pluripotency regulators (Nanog, Sox-2, and Fox-D3) Oct-4 needs to undergo extensive demethylation during nuclear reprogramming and failure of such demethylation is associated with inefficient development of cloned somatic cell embryos (61).

Somatic cloning and expression of Oct-4

Byrne et al (62) have shown that nuclei of mouse thymocytes and human blood lymphocytes injected into frog, *Xenopus laevis* oocytes leads to strong expression of Oct-4. Though, the expression or reprogramming of Oct-4 gene after somatic cloning is essential for proper blastocyst and embryonic development, it has been shown that role of blastocyst formation in clones and regional distribution of mRNA for Oct-4 in clonal blastocyst is dependent upon culture environment (63). Similarly, ability to revert mouse interfollicular epidermal basal keratinocytes to more ES cell like state has been proved with the help of transient transfection. Oct-4 transfected keratinocytes expressed Oct-4 target genes Sox-2, Nanog, undifferentiated transcription factor (Utf-1) and Rex-1. These cells get differentiated to neuronal cells when exposed to neuroectodermal differentiation medium (64).

Tumor translationally controlled protein 1 (Tpt-1) a cancer-associated factor is strongly expressed in tumor cells and suppressed in tumor repression. Recently it has also been shown that Tpt-1 activates transcription of Oct-4 and Nanog in transplanted somatic nuclei (65).

Epigenetic regulation of Oct-4: DNA methylation and chromatin modification

Epigenetics is a phenomenon in which modification of genes takes place without changing gene sequence. It includes modifications such as addition of methyl (-CH₃) groups. Especially, histone tail modifications in the form of acetylation, methylation or ubiquitination etc. are the prominent changes. DNA methylation and histone modification help to either activate or repress

genes depending on the stage of development.

De novo methylation and Oct-4 expression

Oct-4 is localized into MHC locus on chromosome 17 of mouse. It has been found that nuclear and chromatin reorganization in MHC-Oct-4 occurs at developmental phases of ES cell differentiation (66). During embryonic development at about 6.5 dpc a wave of de novo methylation occurs which causes silencing of many of the genes (67,68). Oct-4 gene is unmethylated from blastula stage but undergoes de novo methylation and remains modified in all adult somatic tissues. Methylation of Oct-4 compromises its ability to carry out efficient transcription. But, in order to maintain pluripotential state of ES cells it must remain in an unmethylated state. Oct-4 gene harbors a cis-specific demodification element that includes PE sequence. This protects cells from a wave of de novo methylation and helps in maintaining undifferentiated pluripotential state (69) (Fig 2).

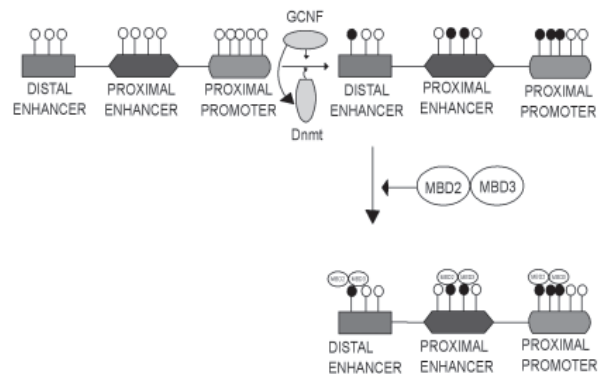


Fig 2: Epigenetic regulation of Oct-4: GCNF, an orphan nuclear receptor binds to proximal promoter of Oct-4. It in turn recruits DNA methyltransferase enzyme which causes DNA methylation at specific sites. This brings about recruitment of methylation specific binding proteins such as MBD2 and MBD3 which bind to methylated regions of Oct-4 regulatory regions. In turn, Oct-4 expression is completely silenced.

Expression pattern in ES and TS cells

In murine blastocyst ICM maintains Oct-4 expression, which contains ES cells while TE cells show complete absence of Oct-4 expression (26). Oct-4 promoter and enhancer region remain hypomethylated in ES cells while hypermethylated in TS cells in vitro. Artificial methylation suppresses Oct-4 expression. When TS cells are cultured with 5-aza-2'-deoxycytidine (DNA methylation inhibitor) and Trichostatin-A (Histone deacetylase inhibitor) activation of Oct-4 gene in TS cells take place.

These cells normally don't express Oct-4. In Dnmt1 n/n mice most of the CpG's in enhancer and promoter region remains unmethylated and Oct-4 expression is aberrantly present. Similarly, Oct-4 enhancer and promoter region is hyperacetylated in ES cells as compared

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with TS cells. Thus, DNA methylation status is closely linked to chromatin structure of Oct-4 gene (70). Similar to mouse ES cells, Oct-4 promoter region of hESC also remains unmethylated. In hESC also Oct-4 transcription is regulated by DNA methylation during early embryogenesis (71). Ferberg et al (72) studied the effect of treatment of EC cell extract on Oct-4 and Nanog expression. Epigenetic reprogramming of OCT-4 and NANOG regulatory regions has been found to occur by EC cell extract, while sequential DNA methylation of Nanog and Oct-4 upstream region has been recorded in human neuronal NT-2 cells during neuronal differentiation (73).

DNA methylation and role of MBPs in Oct-4 silencing

Silencing of gene is normally carried out by DNA methyltransferases. To date three DNA methyltransferases Dnmt1, Dnmt 3a and Dnmt 3b have been reported (74, 75). Dnmt 3a and Dnmt 3b are strong candidates for de novo type DNA methylation (75). Dnmt-1, which contributes to methylation patterns during replication and maintains cell lineage specific methylation patterns in somatic cells, also catalyzes de novo methylation activity in vivo (76, 77). Orphan nuclear receptor (ONR) viz. Germ cell nuclear factor (GCNF) has been found to recruit DNA methyltransferases for Oct-4 silencing (78). DNA methylation of specific genes is followed by recruitment of methylation binding proteins (MBPs) such as MBD-2 and MBD-3. In teratocarcinoma cell line (P19) and ES cells, upon differentiation endogenous GCNF has been found to bind to Oct-4 proximal promoter and recruits MBD-2 and MBD-3. In differentiated GCNF $-/-$ ES cells Oct-4 repression is lost and expression of Oct-4 in somatic cells occurs. Thus, recruitment of MBD-3 and MBD-2 by GCNF links two events, gene specific repression and DNA methylation, which occurs differentially at Oct-4 promoter (79).

Oct-4 regulatory element remains unmethylated in P19 EC cells. These are methylated in somatic cells including cell lines such as embryonic fibroblast (NIH3T3). But, adult somatic cell population has heterogeneous DNA methylation status of regulatory element of mouse Oct-4 gene (80).

Following implantation transcriptional repression of Oct-4 causes pronounced increase in histone H3 methylation of Lys9 mediated by SET containing protein, G9a. It sets stage for local heterochromatinization via binding of HP-1 and is required for de novo methylation at the promoter via enzymes Dnmt3a/3b (81). Thus, it can be seen that Oct-4 is epigenetically regulated by DNA methylation and chromatin modification during embryonic development in pre and post implantation stages.

Regulation of Oct-4 expression

Regulation of Oct-4 expression starts at about 2-cell stage of embryos. Then it is predominantly expressed in ICM of blastocyst. It is concentrated in primitive ec-

toderm and at 7.5 dpc it's solely localized in PGC's. Since, this pattern of Oct-4 expression is crucial for maintaining pluripotency and self-renewal of ES cells careful regulation of its expression is essential.

Proximal promoter: Role in regulation of Oct-4

The upstream region of Oct-4 gene is considered to be the regulatory region, which help in maintaining proper expression levels of Oct-4. Upstream to transcription initiation site ATG it harbors a proximal promoter element, which contains GC rich Sp1 binding site and three hormone response element (HRE) half sites. Promoter also harbors a sequence called as RAREoct which has extensive homology to RA responsive element RARE1 present in Proximal enhancer region. Both these elements show different binding pattern. Retinoic acid (RA) has been found to cause the differentiation of Oct-4 expressing EC cells and subsequent down regulation of Oct-4. RA represses Oct-4 promoter in P19 cells. RAREoct possesses binding sites for nuclear hormone receptor family that consists of RAR (α , β , γ), RXR (α , β , γ) and also ONR family (COUP-TF1, COUP-TFII and EAR-2). RAR binds to all-trans RA while RXR has ligand as 9-cis-RA while ligands for ONR family have not yet been identified.

In undifferentiated P19 cells RAREoct seems to be acting as a binding site for positive regulator (RAR: RXR) of transcription and help in activation of Oct-4 and as negative regulator in P19/RA treated cells. The mutation in RAREoct has been found to abolish RA induced expression. It has been proposed that repression may involve displacement of an activator that binds RAREoct or RA induced repressor may form transcriptionally inactive homo and heterodimers (82-84) (Fig 3).

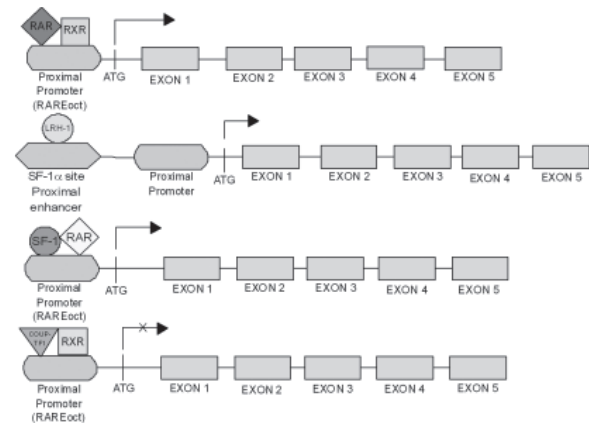


Fig 3: Regulation of Oct-4: Oct-4 is either positively or negatively regulated by several factors. The positive regulators such as RAR: RXR heterodimers, LRH-1 and SF-1: RAR is represented in the figure. They bind to upstream regulatory element such as proximal promoter and mediate Oct-4 expression. The combination of variety of orphan nuclear receptors such COUP-TF1 and RXR as shown in figure bring about down regulation of Oct-4. Apart from COUP-TF1, EAR-2, ARP-1 and COUP-TFII work in same fashion to bring about Oct-4 down regulation.

Orphan nuclear receptor**a) COUP-TF1/TFII, ARP-I AND EAR-2**

Since, RAR: RXR mediate the activation of Oct-4 gene via binding to RAREoct in Oct-4 promoter region. The orphan receptors COUP-TFI, COUP-TF II (ARPI) and EAR2 block this RAR: RXR mediated transactivation by binding as homo or inactive heterodimers with RXR to responsive elements. It is shown that COUP-TF interacts with TFIIB, which forms an integral part of basal transcription machinery and halts the transcription of Oct-4 gene. Thus, negative regulation of Oct-4 expression during RA induced differentiation of ES cells is controlled by two mechanisms viz. deactivation of ES cell specific enhancer and promoter silencing by orphan nuclear receptors. N-terminal of ARP I/COUP-TFII is dispensable for repression and C-terminal harbors silencing region. If the concentration of RAR: RXR is increased the repression mediated by ONR can be overcome (85, 86).

b) Germ Cell Nuclear Factor (GCNF)

GCNF is an orphan nuclear receptor family member, which has been found to repress Oct-4 gene activity by specifically binding within the proximal promoter. GCNF is critical for repressing Oct-4 gene activity as pluripotent stem cells differentiate and in confining Oct-4 expression to germ line (87).

c) Liver Receptor Homologue-1 (LRH-1)

LRH-1 unlike other ONR is required to maintain Oct-4 expression at epiblast stage. LRH-1 expressed in undifferentiated ES cells binds to SF1 response element in proximal and distal enhancer of Oct-4 gene and Oct-4 gene expression is activated. LRH-1 is co localized with Oct-4 in ICM and its disruption leads to loss of Oct-4 expression and early embryonic death (88).

d) Tr2

Small ubiquitin related modifiers (SUMO) are the elements like Ubiquitins except that they are not tagged for protein degradation. They are involved in post translational modification, protein stability, nuclear cytosolic transport and transcriptional regulation. Tr2 an orphan nuclear receptor family member undergoes SUMOylation when it is in abundance. This leads to replacement of co regulators recruited to regulatory regions of its target gene Oct-4. UnSUMOylated Tr2 activates Oct-4, enhancing EC cell proliferation and is localized to premyelocytic leukemia (Pml) nuclear bodies. When it becomes abundant, Tr2 is SUMOylated and is released from nuclear bodies to act as a repressor of Oct-4. Co repressor Rip 140 replaces co activator Pcaf that switches Tr2 from an activator to a repressor. Thus, Tr2 is partitioned into Pml containing and Pml free pools. Thus, SUMOylation dependent partitioning and differential co regulator recruitment contribute to the maintenance of a homeostasis (89).

e) Steroidogenic Factor-1 (SF1)

Another element, which controls the expression of Oct-4, is SF1 transcription factor. There are two putative binding sites for SF1 one in Oct-4 promoter located within RAREoct (SF1 α) while other distally between -193 to -269. SF1 binds to RARE containing SF1 α . The treatment of RA has been found to cause down regulation of both SF1 and Oct-4 as SF1 activates Oct-4 transcription. Since, RAR is an auxiliary protein it forms the interactions to cause the activation by binding to HRE or RAREoct. So, efforts have been taken to study SF1 and RAR interaction. It has been shown that RAR acts in concert with SF1 to activate Oct-4 promoter expression. Unlike RAR, SF1 can't interact with RXR (90).

f) Sox-Oct

A composite Sox-Oct element within distal enhancer of Oct-4 gene is involved in Pou5f1 transcriptional activation in ESC's. Both Oct-4 and Sox-2 bind directly to composite Sox-Oct element in Pou5f1 and Sox2 binding sites in human and mouse ES cells and also control the expression of genes important for maintenance of primitive state (91, 92).

Monti et al (93) studied the effect of gonadotropins on Oct-4 gene expression during mouse oocyte growth. Intraperitoneal injections of PMSG or PMSG followed by hCG were administered. It was found to induce two major increases in Oct-4 expression. One 48 hr after PMSG in oocytes isolated from primordial follicles and second surge following hCG in preovulatory antral oocytes, recruiting oocytes for initiating growth and in selection of oocytes for ovulation.

g) SET domain protein: G9a

Histone methyltransferases, SET domain containing proteins like G9a is a novel mammalian lysine preferring HMTase. It transfers methyl group to lysine residues in histones. G9a mediates irreversible epigenetic inactivation of Oct-4 during early embryogenesis (80). Tsuji et al (94) studied inhibition of Oct-4 expression in mouse preimplantation embryo using morpholino antisense oligonucleotides. Similarly, Tpt1 has been found to activate Oct-4 expression in somatic cell nuclear transfer.

h) GA repeat binding protein- α (GABP- α):

GA repeat binding protein α (GABP- α) is restricted to ES cells and is controlled by STAT3. When GABP- α is knocked down it was found that down regulation of Oct-4 expression occurs along with stimulation of Cdx-2, COUP-TFI, GCNF, which are repressors of Oct-4. Thus, indicating that Oct-4 expression is controlled by GABP- α (95).

Redox Regulation

Another means of regulation of Oct-4 is via redox regulation. Uterine implantation and vascularization increases oxygen exposure of developing embryo. It has been reported that Oct-4 DNA binding is exquisitely

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sensitive to abrogation via oxidation. The reducing enzyme such as Thioredoxin could restore DNA binding activity of Oct-4 (96). Hypoxia leading to expression of HIF-2 α can bind to Oct-4 promoter. Oct-4 has been found to be the target gene of HIF-2 α but not that of HIF-1 α and it can regulate stem cell function which in turn also contributes to HIF-2 α 's tumor promoting activity (97).

Oct-4: A germ cell tumor marker

Recent reports indicate that Oct-4 expression is also present in adult stem cells (98). There are speculations about these stem cells being converted to cancer stem cells which continue to self renew and prove to be lethal. Both normal adult stem cell and cancer stem cell maintain expression of Oct-4. These Oct-4 positive cells might represent the "Cancer stem cells". A strategy to target "Cancer Stem Cells" is to suppress Oct-4 gene in order to cause cells to differentiate (99). Oct-4 pseudogenes localized on human chromosome 10 (pg5) and pseudogene on chromosome 8 (pg1) have been found to be transcribed in cell lines as well as cancer tissues tested. These pseudogenes might be involved in regulation of Oct-4 activity thus might be pertinent to carcinogenesis (100).

Seminomas and Testicular Germ Cell Tumors (TGCTs)

Since, Oct-4 is expressed in germ cells; germ cell origin of metastatic tumors by Immunohistochemical localization of Oct-4 is highly sensitive and specific for diagnosis of seminoma and EC metastatic from testis. We have also shown the expression of c-kit, a protooncogene in spermatogonial stem cells and their knockdown by siRNA led to cell cycle arrest and apoptosis (101). Further our studies revealed that knockdown of c-kit led to differential expression of genes involved in maturation of spermatogonial stem cells (102). Oct-4 has been used as a diagnostic tool for establishing germ cell origin of a metastatic tumor and a potential role of Oct-4 in IHC assessment of carcinoma of unknown primary origin (103). Palumbo et al (104) have reported the expression of PDGF α receptor, Oct-4 and c-kit in human and normal malignant tissues suggesting early diagnosis markers of testicular germ cell tumors.

Rajpert-De Meyts et al (105) studied the developmental expression of Oct-4 in normal and dysgenetic human gonads. Oct-4 was down regulated until about 20 weeks of gestation and thereafter it was rapidly down regulated but persisted in few cells until 3-4 months of postnatal age. It is not detected in testes but ovaries do express Oct-4 in primordial oogonia and then it's down regulated in primary follicles. It has been found that Oct-4 expression is heterogeneous in dysgenetic and intersex cases with Oct-4 positive gonocytes detected till 14 months of age. Neoplastic gonadoblastoma and carcinoma in situ (CIS) expresses abundant Oct-4.

Oct-4 has been suggested to be a tool in identification of primary testicular and Embryonal Carcinoma (EC). It

is also a novel biomarker for dysgerminoma of ovary. Expression of Oct-4 is examined in both dysgerminoma and non dysgerminomatous neoplasms involving ovary. It may also be used in diagnosis of gonadoblastoma. This could help in detection of small foci of metastatic dysgerminoma in extra ovarian sites and may help distinguish dysgerminoma from other primary and metastatic tumors of ovary (100). Along with Oct-4 NANOG, STELLAR and GDF3 are also expressed in both seminoma and breast carcinoma (106). Presence of Oct-4 in bladder cancer led to a clue to involvement of embryonic genes in carcinogenesis (107). Aberrant hypomethylation in many cancers reactivates retrotransposons and selected single copy genes such as cancer-testis antigens. Genes reactivated in this manner are CTCFL/BORIS testis specific chromatin regulator and Oct-4. CTCFL and OCT-4 have been found to resemble cancer testis antigens in being selectively hypomethylated and expressed in male germ cells but differ in lacking significant expression and hypomethylation in prostate carcinomas. Thus, it can be concluded that CTCFL is epigenetically regulated by DNA methylation but not Oct-4 (108).

Conclusion

Since the discovery of Oct-4 POU transcription factor several years ago, our understanding about the role of Oct-4 as a marker for pluripotency has grown considerably. Although our knowledge of Oct-4 has grown substantially, there is still undoubtedly a considerable amount remaining to be discovered. Beyond the role of Oct-4 in pre-implantation development, maintaining pluripotency of ES cells as well as in gonadal formation there is more to be understood on the role of Oct-4 in spermatogenesis. Expression of Oct-4 in spermatogonial stem cells (SSCs) indicates its probable role in early maturation, survival as well as in maintaining undifferentiated state of testicular SSCs. The experiments using systems to turn on and off of Oct-4 in vivo are necessary to determine whether modifications of expression levels determine a major reprogramming of the cell fate during spermatogenesis. There is still a plethora of knowledge left to be discovered regarding the Oct-4 associated factors, target genes which can provide new insights into the understanding of early steps regulating spermatogenesis and the discovery of this knowledge will go long way towards the better understanding about the developmental process. There is also a growing evidence of use of Oct-4 in germ cell tumor detection and further advances in this field would help in the future for establishing germ cell origin of metastatic tumors.

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