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The role of TET proteins during development

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In 2009, a new family of DNA modifying enzymes, the Tet-eleven translocation family was identified as 2-oxoglutarate (2-OG) and Fe(II)-dependent dioxygenases. This family of enzymes comprises three proteins TET1, TET2 and TET3 that share a carboxyl-terminal core catalytic domain consisting of a conserved cysteine-rich domain, a double stranded β-helix domain and binding sites for the cofactors Fe(II) and 2-oxoglutarate. At their amino-terminal region, TET1 and TET3 have a CXXC DNA-binding domain[1]. Interestingly, during evolution, the segment encoding the CXXC domain of TET2 was separated from the region encoding the catalytic domain and is now encoded separately by a neighboring gene, IDAX (also called CXXC4)[2].

All TET proteins are oxygenases capable of oxidizing 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) and further into 5-formylcytosine (5fC) and/or 5-carboxylcytosine (5caC)[1,3]. Since their discovery, dissecting the role of TET enzymes has been the subject of many studies which revealed their implication in DNA demethylation and gene expression regulation[4,5]. Several routes have been described for TET-mediated active and passive DNA demethylation[6]. It was suggested that 5hmC can be diluted through replication due to the low enzymatic activity of DNMT1 on hemi-hydroxymethylated DNA[6]. By an alternative pathway, 5fC and 5caC are targeted by TDG DNA glycosylase and the base-excision-repair machinery to generate unmethylated cytosines[6]. Interestingly, cytosine oxidation products might play a role beyond functioning as transient intermediates in active DNA demethylation, providing an additional layer to the epigenetic code[5]. Indeed oxidized mC were proposed to serve as an anchor for specific epigenetic readers, to modify the recruitment of transcription factors or mC-binding proteins, and to affect RNA polymerase II elongation rate, indicating that TET-mediated oxidation could directly regulate transcription[5]. In addition, studies showed that TETs regulate several target genes by interacting with co-activators such as OGT[7] or co-repressors like Sin3A, PRC2[8-10]. Whether the catalytic activity of TET is required in such cases is not completely well defined. Nevertheless, emerging evidence, including our study[10], reveal catalytic-independent functions of TET enzymes during development[10-12].

TET enzymes in development and diseases

Because TET enzymes are implicated in DNA demethylation, many studies focused on their role at two major waves of DNA methylation remodeling, in the early post-fertilization zygote and during primordial germ cell (PGC) specification.

PGCs, which will give rise to the oocytes or spermatozoids and show high expression of TET1 and TET2, undergo two stages of DNA demethylation. Stage I of DNA demethylation in PGCs is independent from TET enzymes' activity; however, stage II involves their catalytic function[13]. The latter occurs in the mouse particularly at E9.5-E12.5, when genome-wide DNA demethylation, erasure of genomic imprints, and large-scale chromatin remodeling are happening. At those stages, TET1 is suggested

to be critical for the regulation of a set of germline reprogramming-responsive genes involved in gamete generation, meiosis and the maintenance of DNA methylation in gonadal PGCs[14]. Tet3 is highly expressed in oocytes but not in PGCs. All three TETs are detectable in murine sperm cells in considerable amounts[4]. In line with that, TET enzymes are successively expressed in human spermatogenesis and their expression level is pivotal for male fertility[15]. Female mice lacking TET1 have smaller ovary size, reduced oocyte number, decreased fertility and present small litter size[16].

After fertilization, in the zygote, the paternal genome loses its DNA methylation marks rapidly. Several studies suggested that this 5mC loss in the paternal pronuclei is attributed mostly to the activity of the maternal TET3[17,18]. However, the latter is not essential for a normal development because ablation of maternal TET3 is compatible with embryonic development[19]. While maternal TET3 disappears rapidly during the first cleavages of the embryo, the expression levels of *Tet1* and *Tet2* increase during preimplantation development. Indeed, TET1 and TET2 are highly expressed in the inner cell mass (ICM) of the blastocyst and embryonic stem cells (ESCs), the *in vitro* correlate of ICM, where 5hmC is present as well[3,20]. After implantation, while TET2 and TET3 are not detectable, TET1 expression persists in the epiblast and at lower levels in the extra-embryonic ectoderm (ExE) of embryonic stage E6.5. At the latter stage, TET1 plays non redundant functions specific to both lineages[10] Afterwards, TET1 expression diminishes rapidly at E7.5 and persists only faintly in the head folds and neural tube by E8.5. TET3 expression is detectable from E8.5. After axial rotation of the embryo, expression of the three TETs progressively increases in the developing brain of E9.5-10.5[10].

The physiological importance of TETs in development has been investigated using several genetic knockout (KO) mouse models. Studies have shown that constitutive deletion of *Tet3* leads to neonatal lethality[21] and that TET2 deletion results in a normal development[22]. However, reports concerning TET1 KOs have been more variable depending on the mice model used, resulting in some conflicting conclusions. While two studies observed that *Tet1* KO mice are born without any apparent loss *in utero*[23,24], another study also targeting deletion of the C-terminal catalytic function describe partial embryonic lethality, with only 30% of the expected number of *Tet1* knockouts surviving to birth[25]. Because in those studies TET1 KOs were generated by strategies targeting the C-terminal catalytic site, they may have allowed expression of a large N-terminal fragment (~70 kDa) and resulted in hypomorphic phenotypes requiring combined deletion of other TETs[21,26] in order to observe a stronger phenotype. Interestingly, in a *Tet1* gene trap (GT) mice model, investigated by our group and the group of Yi Zhang[10,16], embryonic lethality was observed starting at E8-E9.5 with different penetrance depending on the genetic background (100% in mixed background strains and 60% in C57BL/6J incipient congenic strain). Subsequently, we validated these GT phenotypes in a new mice model that we developed by an independent targeting strategy in which the lacZ reporter cassette is inserted immediately in-frame downstream of the ATG start codon[10]. The latter model confirmed that TET1 can be essential for embryonic development. Briefly, our analysis[10] of those two mice models with ablation in the 5’ coding sequence suggests that while the catalytic domain of TET1 can be dispensable, further regulation by its N-terminal domain is critical for a proper development. This clarifies why previously reported studies showed

little or no effect of TET1 loss on embryonic development.

The functions of TET enzymes are not limited to early embryonic developmental stages and to embryonic stem cells (ESCs). Studies point towards an important role of these proteins in the brain[26] and hematopoietic lineages[5]; in line with that, they seem to be implicated in several diseases related to those tissues (i.e. Leukemia[5], Alzheimer disease, Huntington's disease[27]…).

In post-natal development, TET1 and 5hmC are abundant in the brain and TET1 has been investigated as an important contributor to brain development[28]. Recent studies indicated that TET1 deficiency decreases 5hmC levels in brain[29,30], resulting in impaired hippocampal neurogenesis[31]. Abnormal TET1 expression leads to neuronal activity–regulated gene downregulation, synaptic plasticity alterations, and cognitive impairments[32,33]. Yet, little is known about the functions of TET1 in the early embryonic development of neuronal and brain tissues.

Finally, the disruption of TET1 and TET2 in adult hematopoietic tissues leads to increased hematopoietic stem cell (HSC) function[34,35], variable degrees of myeloid and lymphoid alterations and defects in terminal hematopoietic lineage differentiation. For example, *Tet2*$^{-/-}$ mice develop leukemia spontaneously[35]. In humans, somatic alterations and mutations in *TET2* are observed in a wide range of hematological disease[5]. Even though somatic alterations of *TET1* or *TET3* are less abundant than TET2 in patients with hematological diseases, murine models combining double mutations of TET enzymes are prone to developing late-onset B-cell lymphoma[34] (TET1/2 KO) or a rapid and fully penetrant myeloid leukemia within 7 weeks[36] (TET2/3). It is important to mention that the implication of TET enzymes in malignancies seems not to be limited to hematological cancers. Indeed, mutations of TET enzymes and modifications in 5hmC levels have been described in other cancers (i.e. gastric, prostate, liver, lung, breast cancer…)[5]. However, their direct role in such cancers still needs to be investigated.

Since their discovery, many facets of TET enzymes roles in pre- and post-natal development and their involvement in many diseases have been revealed. However, many other aspects still need to be dissected and several questions need to be answered. For example, it is important to uncover the mechanisms of actions of TET enzymes, their targeted pathways and their initiating role underlying those diseases. Moreover, it is essential to understand the impact of any deregulations in TET functions acquired during embryonic development on post-natal health. Regarding the latter, our recent study[10] revealed many changes in DNA methylation at pre-gastrulation stages upon loss of TET1 (For details see our earlier article published in PDJ). Interestingly, only 40% of those changes were present at differentially expressed genes at that stage; however, the majority was associated with genes involved in diseases (i.e. Alzheimer, Huntington…). Even though the catalytic functions of TET1 at the early pre-streak embryo seem to be “dispensable” for embryonic development, they may be the basis of many post-natal diseases.

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