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TET mediated epigenetic regulation of iNKT cell lineage fate choice and function

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ABSTRACT

During the last years, intensive research has shed light in the transcriptional networks that shape the invariant NKT (iNKT) cell lineage and guide the choices towards functionally distinct iNKT cell subsets (Constantinides and Bendelac, 2013; Engel and Kronenberg, 2014; Gapin, 2016; Kim et al., 2015). However, the epigenetic players that regulate gene expression and orchestrate the iNKT cell lineage choices remain poorly understood. Here, we summarize recent advances in our understanding of epigenetic regulation of iNKT cell development and lineage choice. Particular emphasis is placed on DNA modifications and the Ten Eleven Translocation (TET) family of DNA demethylases.

1. Introduction

Invariant NKT (iNKT) cells are a small subset of T cells that express an invariant Va14Ja18 TCR chain in mice and a limited number of Vβ TCR chains (Vβ2, Vβ7, Vβ8.1, Vβ8.2, Vβ8.3) (Bendelac et al., 2007). iNKT cells have some unique features among the T lymphocytes since unlike the conventional T cells that recognize peptide antigens they do not see peptides in the context of MHC but self and foreign lipid antigens presented by an MHC class I like molecule, CD1d (Bendelac et al., 2007; Brennan et al., 2013; Engel and Kronenberg, 2012). Only hematopoietic cells, such as macrophages, granulocytes, dendritic cells, T cells and B cells, express CD1d (Brossay et al., 1997; Roark et al., 1998). iNKT cells maintain a poised effector state and can respond to proinflammatory cytokines and danger signals in an innate like manner (Brigl et al., 2003; Brennan et al., 2013, 2011). They acquire their functional properties in the thymus and thus they can rapidly elicit an immune response in the organs where they reside. Thus, they are considered to bridge innate and adaptive immunity.

2. iNKT cell specification: stages versus subsets

iNKT cells develop from the DP cells that express CD1d and present lipid antigens recognized by invariant TCR of iNKT precursors (Egawa et al., 2005; Gapin et al., 2001). iNKT cells are selected by agonists (Hogquist and Jameson, 2014). The strong TCR signal that governs their positive selection induces the expression of Egr1 and Egr2 (Seiler et al., 2012), which in turn induces the expression of the transcription factor PLZF (Seiler et al., 2012). PLZF seals the iNKT cell lineage fate (Kovalovsky et al., 2008; Savage et al., 2008). Based on the expression of surface markers these cells have been traditionally subdivided in subsets: stage 0 iNKT cells are $CD24^{high}$, $CD44^{-}$ and NK1.1- and can highly proliferate as they express Myc (Benlagha et al., 2002; Dose et al., 2009). Stage 1 iNKT cells downregulate CD24. Stage 1 is very important for self-expansion (from stage 0 to stage 1) and memory acquisition (during the transition from stage 1 to stage 2). During stage 2 the cells upregulate CD44, expressing thus high levels of this marker, while they remain negative for NK1.1 (Bendelac et al., 2007). These cells also have a highly proliferative capacity as they express high levels of Lef1 and Myc (Carr et al., 2015). Stage 3 iNKT cells upregulate NK1.1 expression and mainly secrete IFNγ (Bendelac et al., 2007).

Recently, the delineation of iNKT cell subsets based on transcription factor expression profiles has been introduced and reflects the heterogeneity of these cells more accurately (Constantinides and Bendelac, 2013; Kim et al., 2015; Lee et al., 2013; Engel and Kronenberg, 2014; Gapin, 2016) (Fig. 1). Based on this approach iNKT cells are subdivided to mirror the helper T cell subsets: thus, NKT2 cells express the transcription factor Gata3, are $CD4^{+}$ and potently secrete IL-4 resembling Th2 cells, NKT17 cells express the lineage specifying factor RORγt,

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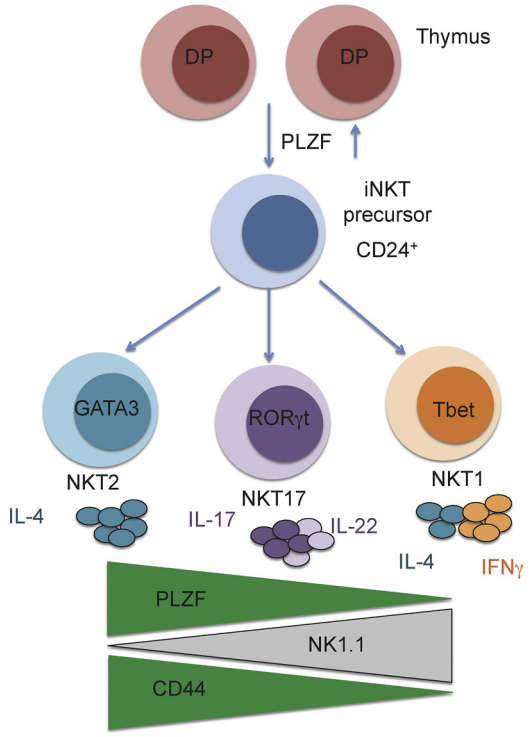

Fig. 1. Representation of iNKT cell subsets. DP cells that recognize antigen presented by CD1d molecules can give rise to the iNKT cell precursor, which expresses the transcription factor PLZF and has high levels of CD24. Distinct iNKT cell subsets develop in the thymus; NKT2 subset expresses GATA3 and secretes IL-4. NKT17 cells express RORγt and secrete largely IL-17 and in less extent IL-22. The NKT1 subset expresses Tbet and secretes mainly IFNγ as well as IL-4. Also the expression levels of PLZF and surface markers NK1.1 and CD44 are depicted.

don't express CD4 and secrete IL-17 like Th17 cells whereas the fate of NKT1 cells is sealed by T-bet that turns on the expression of IFNγ just like in Th1 cells (Constantinides and Bendelac, 2013; Lee et al., 2013). The advantage of this approach is that it enables us to distinguish NKT2 and NKT17 cells that were previously considered to embody NKT stage 2 (Lee et al., 2013) (Fig. 1). These subsets migrate to peripheral organs in a tissue specific manner; NKT1 cells are predominant in the spleen, NKT2 in the lung whereas NKT17 are more prevalent in the lymph nodes (Lee et al., 2015). So, iNKT cells are in many cases already localized in tissues and can be tissue-resident showing almost no recirculation (Fan and Rudensky, 2016; Lynch et al., 2015). Notably, recent studies took advantage of the advances in next generation sequencing that enables gene expression profiling and generation of chromatin maps to shed light in the transcriptional heterogeneity of each subset (Engel et al., 2016; Georgiev et al., 2016; Lee et al., 2016). However, the epigenetic regulation of these cells remains poorly understood.

3. Epigenetic mechanisms of gene regulation

In 1942, Waddington introduced the concept of "epigenetics" as changes that occur in the phenotype but that were not accompanied by changes in the genotype. It is now well established that epigenetic mechanisms mediate the inheritance of gene expression programs by causing changes in chromatin while maintaining the DNA sequence unaltered (Allis and Jenuwein, 2016). Epigenetic mechanisms of gene regulation should meet at least one of the following criteria: the signal must be propagated through cell division, it should be inherited to the daughter cells and it should impact gene expression (Bonasio et al., 2010). Dynamic changes in chromatin modifications (Zhou et al., 2011) and DNA modifications(Pastor et al., 2013; Smith and Meissner, 2013) constitute major epigenetic mechanisms. In the present review, we focus on dynamic changes in DNA methylation mediated by the Ten Eleven Translocation (TET) family of proteins.

3.1. DNA methyltransferases

3.1.1. DNA modification

DNA methylation of cytosine is ensured by the catalytic activity of the family of DNA methyltransferases (DNMTs) (Goll and Bestor, 2005) DNMT1, DNMT3a, DNMT3b. The concept was that 5 methylcytosine (5mC) can be passively diluted via replication in mammalian cells. In somatic cells, 5mC is almost exclusively found in the CpG sequence context (Lister et al., 2009). Genome wide studies using bisulfite sequencing to assess cytosine methylation have established that highly transcribed genes have lowly methylated CpG promoters whereas silenced non-transcribed genes show high levels of cytosine methylation in the CpG context of their promoters (Laurent et al., 2010; Lister et al., 2009). The role of intragenic methylation is rather obscure. Recently, it has been suggested that methylation in gene bodies prevents aberrant intragenic transcription (Neri et al., 2017). Methylation of repetitive DNA sequences, found close to centromeres, is instrumental in the maintenance of genomic integrity. DNMT1 in complex with UHRF1 recognize hemimethylated DNA (Arita et al., 2008; Avvakumov et al., 2008).

3.2. TET proteins; DNA demethylation

TET proteins are 2-oxoglutarate- and Fe(II)-dependent dioxygenases that catalyze the hydroxylation of 5mC to 5-hydroxymethylcytosine (5hmC) in DNA (Tahiliani et al., 2009) and further downstream oxidized products (oxi-mCs) 5 formylcytosine (5fC) and 5 carboxylcytosine (5caC) (He et al., 2011; Ito et al., 2011). TET proteins mediate "active" (replication-independent) DNA demethylation, achieved through excision of 5fC and 5caC by thymine DNA glycosylase (TDG) followed by replacement with an unmethylated cytosine through base excision repair (Pastor et al., 2013; Branco et al., 2012). Notably, the majority of 5hmC is passively diluted via replication (Nestor et al., 2015; Tsagaratou et al., 2014) (Fig. 2). Additionally to their role in mediating DNA demethylation, the oxidative derivatives of TET function -5hmC as well as the less abundant 5fC and 5caC- are also distinct and stable epigenetic marks that can recruit specific readers and impact genomic integrity, DNA repair and transcriptional elongation (Mellen et al., 2012; Spruijt et al., 2013; Cimmino and Aifantis, 2017; Tsagaratou et al., 2017b; Wu and Zhang, 2017).

3.3. TET family of proteins

Suggesting a conserved regulatory role in DNA methylation, representatives of the TET/JBP superfamily exist in every metazoan organism (Iyer et al., 2009; Pastor et al., 2011). In mammalian cells, three members of the TET family of proteins have been identified; TET1, TET2 and TET3. They arose from a common ancestral gene that underwent triplication in jawed vertebrates. All three TET proteins have a common catalytic domain (Fig. 3) and this shared enzymatic activity allows the oxidization of 5mC to downstream oxi-mCs (He et al., 2011; Ito et al., 2010, 2011). The C-terminal catalytic domain consists of a cysteine rich and a double stranded β-helix (DSBH) DNA binding domain and has iron and oxo-glutarate dependent activity. The DSBH DNA binding domain brings together iron, α-ketoglutarate and 5mC for oxidation. The cysteine rich domain wraps around the DSBH and stabilizes the interaction of TET with the DNA. Importantly, the interaction of TET with the DNA does not involve the methyl group and this permits TET proteins to catalyze the oxidation of different modified cytosines (Hu et al., 2013).

Moreover, TET1 and TET3 share an N-terminal CXXC DNA binding