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Short communication

# Sonic Hedgehog regulates thymic epithelial cell differentiation

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ABSTRACT

Sonic Hedgehog (Shh) is expressed in the thymus, where it regulates T cell development. Here we investigated the influence of Shh on thymic epithelial cell (TEC) development. Components of the Hedgehog (Hh) signalling pathway were expressed by TEC, and use of a Gli Binding Site-green fluorescence protein (GFP) transgenic reporter mouse demonstrated active Hh-dependent transcription in TEC in the foetal and adult thymus. Analysis of Shh-deficient foetal thymus organ cultures (FTOC) showed that Shh is required for normal TEC differentiation. Shh-deficient foetal thymus contained fewer TEC than wild type (WT), the proportion of medullary TEC was reduced relative to cortical TEC, and cell surface expression of MHC Class II molecules was increased on both cortical and medullary TEC populations. In contrast, the Gli3-deficient thymus, which shows increased Hh-dependent transcription in thymic stroma, had increased numbers of TEC, but decreased cell surface expression of MHC Class II molecules on both cortical and medullary TEC. Neutralisation of endogenous Hh proteins in WT FTOC led to a reduction in TEC numbers, and in the proportion of mature Aire-expressing medullary TEC, but an increase in cell surface expression of MHC Class II molecules on medullary TEC. Likewise, conditional deletion of *Shh* from TEC in the adult thymus resulted in alterations in TEC differentiation and consequent changes in T cell development. TEC numbers, and the proportion of mature Aire-expressing medullary TEC were reduced, and cell surface expression of MHC Class II molecules on medullary TEC was increased. Differentiation of mature CD4 and CD8 single positive thymocytes was increased, demonstrating the regulatory role of Shh production by TEC on T cell development. Treatment of human thymus explants with recombinant Shh or neutralising anti-Shh antibody indicated that the Hedgehog pathway is also involved in regulation of differentiation from DP to mature SP T cells in the human thymus.



## 1. Introduction

The thymus provides a specialised environment for the production of T cells. Thymic epithelial cells (TECs) are an essential component of the thymic stroma, and are required to support T cell development. Two broad categories of TEC, which are believed to arise from a common progenitor, have been defined by their localisation, function and cell surface markers [1,2]. Cortical(c)TEC provide Dl4 for T cell fate specification, and present MHC + peptide ligands for positive selection. They are defined as EpCam1$^+$, CD40$^+$, CD205$^+$, Ly51$^+$ and MHCII$^+$, and express genes for antigen presentation, including *Cathepsin-L*, *Prss16* and *β5t*. Medullary (m)TEC are specialised for negative selection, and are defined as surface EpCam$^+$, CD40$^+$, CD205$^-$, Ly51$^-$ and MHCII$^+$ cells that react with the lectin UEA-1. Some mTEC express the *Aire* gene and *Cathepsin-S*, facilitating expression and presentation of Tissue Restricted Antigens for induction of tolerance. While TEC provide multiple essential signals for T cell development, they also require signals from thymocytes for their maturation.

Aire function in mTEC is essential for the induction of tolerance to self in both humans and mice, and Aire mutation leads to profound multi-organ autoimmunity [3,4]. Other factors which regulate mTEC differentiation and function are also likely to influence self-tolerance, but currently TEC differentiation is not well understood. To date, only a few factors have been identified that are required for TEC differentiation, such as the transcription factor *FoxN1*, which when expressed ectopically can programme other lineages to a TEC fate [5].

During foetal thymus ontogeny, TEC differentiation has been defined in terms of cell surface expression of CD40 and CD205. The TEC progenitor population, which is bipotential for cTEC and mTEC

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is contained within the CD40$^{low}$CD205$^{low}$ population [6,7]. Further gradual acquisition of CD40 and increase in CD205 expression gives rise to a transitional progenitor, able to differentiate into either functional cTEC, or into mature mTEC that lose CD205 expression and acquire the expression of mTEC characteristic markers, such as Aire [8,9]. These observations are consistent with another study which showed that Aire-expressing mTEC originate from β5t$^{+}$ precursors, a molecule expressed in mature cTECs and not in other cell types [10]. Although these markers have proved useful for investigating TEC development, the lineage relationship between mTEC and cTEC populations and the factors that drive the progression from bipotent progenitors, through transitional intermediates, to mature TEC are not well understood in foetal or post-natal thymus [1].

Interactions between TEC and thymocytes have been shown to promote the terminal differentiation of TEC lineages, particularly mTEC, but fate specification to either lineage is believed to occur independently of signals from thymocytes [11]. Relatively few secreted factors or cell–cell interactions have been identified that regulate TEC differentiation, although members of the tumour necrosis factor receptors super family (TNFRSF), including RANK (TNFRST11a), and CD40 and TGF-β are required for normal thymus medulla development, growth and function [12–14].

Here, we investigate the role of Sonic Hedgehog (Shh) in the regulation of TEC development. Shh is one of three mammalian Hedgehog proteins (Shh, Indian hedgehog (Ihh) and Desert Hedgehog (Dhh)) which share a common signalling pathway. Hedgehog proteins signal by binding to their cell surface receptor Patched1 (Ptch1), and this binding releases Ptch1's repression of Smoothened (Smo), allowing Smo to transduce the Hh signal. At the end of the signalling pathway are the Hh-responsive transcription factors, Gli1, Gli2 and Gli3. *Gli1* is itself an Hh-target gene, and encodes an activator of transcription, whereas Gli2 and Gli3 can be processed to function as transcriptional activators (in the presence of Hh pathway activation) or transcriptional repressors (in the absence of Hh pathway activation). Gli2 is required to intiate the Hh signal, and functions largely as a transcriptional activator *in vivo*, whereas Gli3 functions predominantly as a transcriptional repressor *in vivo*, and can act to repress *Shh* transcription (by repression of an intermediate transcriptional activator) [15]. In fact, in many tissues, Shh and Gli3 have opposing functions, with Shh-deficiency and Gli3-deficiency giving opposing phenotypes [15].

Hedgehog proteins are expressed in the thymus [16–18], and signal to developing T cells to promote differentiation and proliferation of early thymocyte progenitors [19,20]. In both mouse and human studies, Hh signalling has been shown to negatively regulate pre-TCR induced differentiation from CD4$^{-}$CD8$^{-}$ double negative (DN) to CD4$^{+}$CD8$^{+}$ (DP) cell [17,21–23]. In addition, in mouse studies, Shh has been shown to inhibit TCR-induced differentiation from DP to mature CD4 and CD8 single positive (SP) thymocytes [24–27]. Sonic hedgehog (Shh) is expressed by thymic stromal cells, and immunofluorescence has located these cells

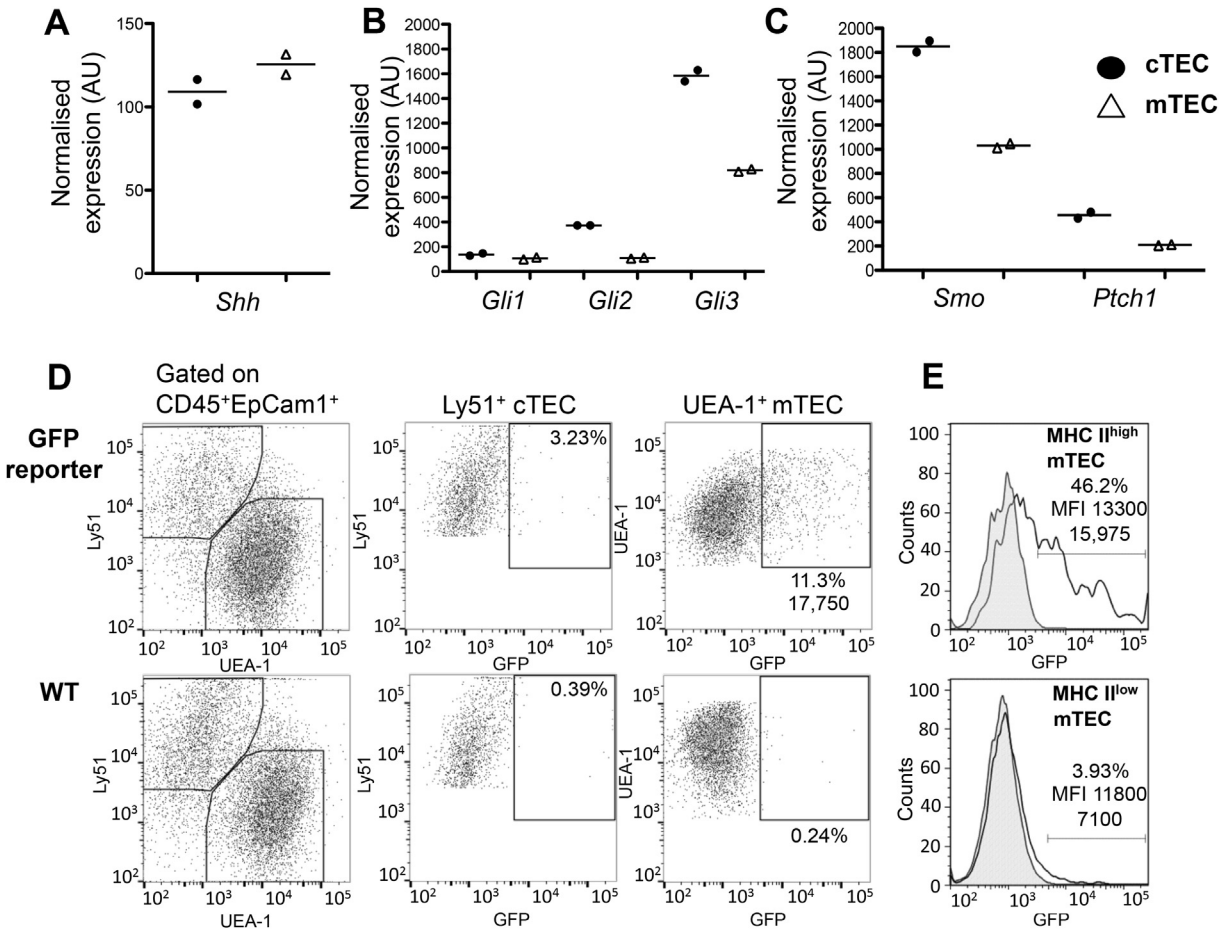


**Fig. 1. Hedgehog signalling is active in thymic epithelial cells from adult mice.** (**A**–**C**) Gene expression by microarray [41] of components of the Hh signalling pathway from sorted cTEC and mTEC extracted from 4 week-old mice. (**D**) Hh signalling in TEC measured by Gli-mediated GFP expression using a reporter transgenic (GBS-GFP-transgenic). (**E**) GFP expression in mature MHCII$^{high}$ and immature MHCII$^{low}$ mTEC. Numbers within plots indicate percentage of GFP positive cells and mean fluorescence intensity (MFI). (**D-E**). Data representative of three independent experiments.

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