

Lack of Evidence for Activation of the Hedgehog Pathway in Psoriasis

Johann E. Gudjonsson¹, Abhishek Aphale¹, Marina Grachtchouk¹, Jun Ding², Rajan P. Nair¹, Timothy Wang¹, John J. Voorhees¹, Andrzej A. Dlugosz¹ and James T. Elder^{1,3}

Recent reports have suggested that the hedgehog (Hh) pathway is activated in lesional psoriatic skin, and that treatment with the Hh pathway antagonist cyclopamine may lead to rapid resolution of the disease. To assess Hh pathway activity in psoriasis, we isolated RNA from lesional and uninvolved skin of 58 psoriatic patients, and from 63 normal control subjects, and subjected these samples to global gene expression profiling on Affymetrix HU133 Plus 2.0 gene arrays. We were especially interested in Hh target genes (*PTCH1* and *GLI1*), whose expression is elevated in response to Hh signaling. The microarray data demonstrated downregulation of *PTCH1* expression in uninvolved and lesional skin (1.1-fold and 2-fold, respectively; $P < 0.0001$). Additionally *GLI1* mRNA was downregulated in lesional skin (1.7 fold; $P < 0.05$). No significant changes were observed between lesional and uninvolved skin for the Hh ligands or Smoothened. Quantitative PCR confirmed these findings. *In situ* hybridization for *GLI1* and *PTCH1* was positive in basal cell carcinoma tumor cells, but was negligible in uninvolved or lesional psoriatic skin. The absence of elevated Hh target gene expression in lesional psoriatic skin indicates that the Hh pathway is not activated in this disease, raising questions regarding the proposed use of Hh antagonists as antipsoriatic agents.

Journal of Investigative Dermatology (2009) **129**, 635–640; doi:10.1038/jid.2008.266; published online 28 August 2008

INTRODUCTION

Psoriasis is a common chronic inflammatory skin disease characterized by complex alterations in epidermal growth, differentiation, and multiple, immunological, and vascular abnormalities. Psoriasis was historically considered to be a primary disorder of keratinocytes, but over the past two decades it has been firmly established that psoriasis is an immune disorder mediated by activated T cells (Gudjonsson *et al.*, 2004; Liu *et al.*, 2007). How activated T cells mediate the altered differentiation and hyperproliferation of keratinocytes and other changes observed in psoriasis is still unknown but is thought to involve a highly complex, yet incompletely understood, interaction of multiple cytokines and growth factors with multiple cellular effectors present within the psoriatic lesion. Additionally, the intercellular signaling pathways mediating these changes remain to be fully elucidated, although reports have indicated involvement of the signal transducer and activator of transcription 1

(Bowcock *et al.*, 2001), signal transducer and activator of transcription 3 (Sano *et al.*, 2005), mitogen-activated protein kinase (Johansen *et al.*, 2005b), activator protein 1 (Johansen *et al.*, 2004), and the NF- κ B pathways (Lizzul *et al.*, 2005; Johansen *et al.*, 2005a).

Recent reports have suggested that the hedgehog (Hh) pathway is activated in lesional psoriatic skin (Kuenzli *et al.*, 2004; Tas and Avci, 2004; Endo *et al.*, 2006; Meth and Weinberg, 2006), and that pharmacological inhibition of this pathway using cyclopamine may lead to rapid resolution of the disease (Kuenzli *et al.*, 2004; Tas and Avci, 2004; Meth and Weinberg, 2006). The Hh signaling pathway is one of the major signaling pathways involved in embryonic development (Ingham and Placzek, 2006). During physiologic Hh signaling, Hh proteins bind to the cell surface receptor Patched (PTCH1), thereby releasing Smoothened (SMO) from PTCH-mediated inhibition. SMO activation then triggers a series of intracellular events, culminating in alterations in gene expression mediated by the Gli family of transcription factors (GLI1, GLI2, and GLI3; Ruiz i Altaba *et al.*, 2007; Figure 1). The transcripts for PTCH1 and GLI1 are reliable markers for both physiologic and pathologic Hh signaling activity as they are consistently induced when the Hh pathway is activated (McMahon *et al.*, 2003; Hutchin *et al.*, 2005). This pathway has been shown to be a crucial regulator of hair follicle growth and sebaceous gland biology (Allen *et al.*, 2003; Niemann *et al.*, 2003). On the other hand, sustained activation of the Hh pathway appears to be the driving force for basal cell carcinoma (BCC) development (Daya-Grosjean and Couve-Privat, 2005).

¹Department of Dermatology, University of Michigan Medical Center, Ann Arbor, Michigan, USA; ²Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA and ³Ann Arbor Veterans Affairs Health System, Ann Arbor, Michigan, USA

Correspondence: Dr Johann E. Gudjonsson, Department of Dermatology, 1910 Taubman Center, 1500 E. Medical Center Drive, University of Michigan, Ann Arbor, Michigan, USA. E-mail: johanng@med.umich.edu

Abbreviations: BCC, basal cell carcinoma; CCN, cyclin; DHH, desert hedgehog; Hh, hedgehog; IHH, Indian hedgehog; PTCH, Patched; QT, quantitative; SHH, Sonic hedgehog; SMO, Smoothened

Received 27 April 2008; revised 26 June 2008; accepted 30 June 2008; published online 28 August 2008

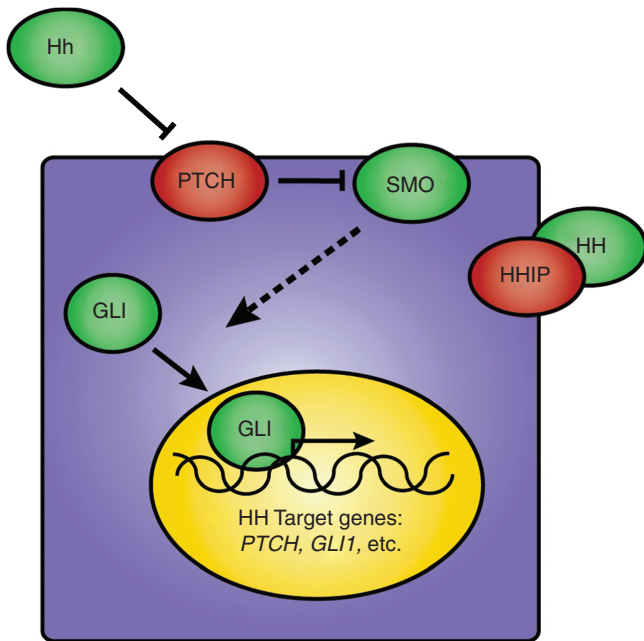


Figure 1. Highly simplified diagram illustrating key components of the Hh signaling pathway. Signaling activators are indicated in green, signaling inhibitors in red. In the absence of Hh ligands (SHH, IHH, or DHH), PTCH blocks the function of the key signaling effector, SMO. Hh ligands block the inhibitory effects of PTCH, leading to derepression of SMO and activation of intracellular signaling. HH pathway activation leads to reprogramming of gene expression via the GLI family of transcription factors (GLI1, GLI2, and GLI3). Hh target genes, in addition to PTCH and GLI1, include the Hh inhibitor protein HHIP, cyclins CCND1 and CCND2, and PTCH2.

The involvement of the Hh pathway in psoriasis was first suggested by the marked and rapid improvement observed in a clinical trial, in which 31 individual psoriatic lesions in seven patients, all with an established diagnosis of plaque or guttate psoriasis, were treated with topical cyclopamine (Tas and Avci, 2004). Furthermore, the authors asserted that topical cyclopamine was more effective than the potent topical steroid clobetasol-17 propionate, a typical first-line therapy (Tas and Avci, 2004). Cyclopamine is a steroid alkaloid that acts as an inhibitor of the Hh pathway by binding directly to SMO and thus blocking activation of the pathway. Cyclopamine is a potent teratogen that can lead to severe fetal malformations including cyclopia (holoprosencephaly; Belloni *et al.*, 1996; Roessler *et al.*, 1996). With application of cyclopamine to the lesional skin marked improvement was seen within 24 hours with near complete clinical and histological clearance observed in 96 hours (Tas and Avci, 2004). In another recent study it was demonstrated by immunohistochemistry that GLI1 was overexpressed in lesional skin whereas no expression was detected in either uninvolved or control skin (Endo *et al.*, 2006). Taken together, these data suggested that Hh pathway activation is proximal to other events in the pathogenesis of psoriasis and that therapeutic manipulation of this pathway may lead to rapid and possibly sustained improvement of psoriasis.

Despite these data, detailed evaluation of this pathway, and its activation status, in psoriasis has been lacking.

We have collected large datasets on gene expression in psoriasis to evaluate and dissect the pathogenic basis of this enigmatic disease. Here, we investigate the principal components of the Hh pathway in lesional, uninvolved, and normal skin and expression of its downstream signature target genes.

RESULTS

Microarray data

Several of the genes involved in the Hh Pathway showed modest changes in expression among control, uninvolved, and lesional skin (Figure 2), but in no case did we detect evidence for activation of Hh signaling activity in psoriasis. Expression of the *PTCH1* gene was only slightly downregulated in uninvolved psoriatic skin (89% of control, $P < 0.0001$) with more pronounced downregulation in lesional skin (51% of uninvolved, $P < 0.0001$; Table 1), compared to control skin. No changes were observed in the expression levels of the *PTCH2* gene. Both *GLI1* and *GLI2* were significantly downregulated in lesional psoriatic skin (60 and 68% of uninvolved, $P < 0.05$ and $P < 0.0001$, respectively; Table 1). Cyclin D1 (*CCND1*) was significantly downregulated compared to uninvolved (2.2-fold, $P < 0.0001$) in lesional psoriatic skin whereas *CCND2* was upregulated (1.5-fold, $P < 0.0001$), consistent with previously published data (Belso *et al.*, 2008). Additionally, *CDC2* and *CCNB1* were also upregulated relative to uninvolved skin (1.5-fold, 3.3-fold, and 5.6-fold, respectively; $P < 0.0001$). The Sonic hedgehog (*SHH*) and the Indian hedgehog (*IHH*) gene expressions were minimally upregulated in uninvolved psoriatic skin relative to control (1.06- and 1.05-fold, $P < 0.05$) whereas no change was observed for the desert hedgehog (*DHH*) gene. No significant changes were observed between lesional and uninvolved skin for the Hh ligands or SMO (Table 1). Molecular network mapping performed by ingenuity pathway analysis demonstrated complete lack of activation of the Sonic hedgehog pathway (data not shown).

Real-time quantitative PCR data

For confirmation of the microarray data, quantitative (QT) real-time PCR was performed on RNA isolated from 10 control skin biopsies, 10 paired uninvolved and lesional psoriatic skin biopsies, and 12 BCC samples, which served as positive controls for Hh pathway activation. The expression of the principal Hh pathway factors, *GLI1* and *PTCH1*, were significantly lower in lesional psoriatic skin than in BCC ($P < 0.001$; Figure 3). Furthermore, expression of these two genes was lower in lesional skin compared to uninvolved skin ($P = 0.10$ (*GLI1*), $P < 0.05$ (*PTCH1*)). Expression of *PTCH2* and *HHIP* were significantly lower in lesional, uninvolved, and control skin compared to BCC ($P < 0.01$; not shown), whereas the expression of keratin 1b and *CCND1* was decreased in both lesional skin and BCCs compared to normal and uninvolved skin ($P < 0.001$, $P < 0.01$, respectively; data not shown). *CCND2* and myc myelocytomatosis viral related oncogene (neuroblastoma derived) expression levels were significantly lower in BCC

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