Transcription factor Sp1 activates involucrin promoter activity in non-epithelial cell types

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The gene for human involucrin (hINV) is selectively expressed in stratifying epithelial cells lining external body surfaces. Previously, we characterized the hINV promoter 5' distal regulatory region (DRR) located between nt –2473 and –2088 upstream of the transcription start site. This region is required for optimal hINV gene expression. The DRR contains weak and strong activator elements. The strong activator comprises AP1- and Sp1-binding sites that combine to drive high-level promoter expression in human keratinocytes. Here we show that the hINV promoter is expressed in a cell-specific manner *in vitro* and that the DRR contains elements that are partly responsible for cell-type-specific expression of hINV. hINV promoter activity is barely detectable in 3T3 fibroblasts or HEK-293 human embryonic kidney cells. Reporter plasmids containing the full-

length promoter or the isolated DRR can, however, be activated in 3T3 and HEK-293 cells by co-transfection with a plasmid encoding the transcription factor Sp1. Consistently with the lower hINV promoter activity, immunoblotting studies indicate that Sp1 protein levels are lower in 3T3 and HEK-293 cells than in human epidermal keratinocytes. Increased Sp1 protein in transfected 3T3 cells and HEK-293 cells correlates with increased promoter activity. In addition, Sp1 transfection activates the expression of the endogenous gene for hINV in HEK-293 cells. These studies suggest that Sp1 might have a role in cell-specific expression of hINV.

Key words: differentiation, epidermis, gene regulation, keratinocyte, Sp1.

INTRODUCTION

Human epidermis is a keratinizing squamous epithelium consisting of several distinct layers [1]. The basal cell layer is located adjacent to the dermis and consists of undifferentiated proliferating cells. During the process of terminal differentiation, the keratinocytes lose their ability to proliferate and migrate from the basal layer to the superficial layers while undergoing a co-ordinated series of morphological, biochemical and genetic changes. The terminal differentiated cell, or corneocyte, is a flattened dead cell that consists of a network of cytokeratin filaments surrounded by an insoluble envelope of heavily cross-linked protein [1–7]. The envelope precursors and transglutaminase, the enzyme activity responsible for the assembly of the envelope, must be expressed at the correct time and level during the differentiation process for proper envelope formation. Involucrin, a component of the cornified envelope, is specifically expressed in the suprabasal layers of the epidermis [8–12]. It accumulates in the spinous and granular layers as a non-cross-linked precursor and becomes covalently cross-linked to other proteins to form the cornified envelope during the final stages of keratinocyte differentiation [13].

Transgenic mouse studies indicate that the involucrin promoter is expressed in a cell-type-specific manner *in vivo* [14]. Our previous studies indicate that the distal regulatory region (DRR), which includes nt –2473 to –2088 upstream of the transcription start binding site, accounts for one-half of the basal and phorbol ester-induced activity of the human involucrin (hINV) promoter [15]. This region contains weak and strong activator elements [16]. The strong activator element is composed of AP1- (AP1-5) and Sp1-binding sites separated by a single nucleotide [16]. AP1

factors junB, junD and Fra-1 interact with the AP1-5-binding site, whereas Sp1 (but not Sp2, Sp3 or Sp4) binds to the Sp1-binding site [15,16]. The Sp1-binding site is required for maximal binding of AP1 factor to the AP1-5-binding site; both binding sites are required for maximal activation of the hINV promoter [16].

Little is known about mechanism responsible for the cell-type-specific and differentiation-specific expression of epidermal genes. Research into the epithelium-specific transcription of cellular genes, including keratins 5, 7, 4, 14 and 18, and E-cadherin, has led to the complementary observation that these promoters are regulated by ubiquitous transcription factors [17–23], suggesting a model of combinatorial regulation by ubiquitous factors (and cofactors) to achieve cell-type-specific expression. Sp1 has a role in the keratinocyte-specific expression of the TG3, K3 and K5 genes [24–26]. In addition, Sp1 protein levels are known to increase during the differentiation of keratinocytes [27], suggesting that Sp1 protein could contribute to epithelium-specific gene expression. Our present studies suggest a role for Sp1 as a mediator of cell-type-specific expression of the hINV promoter.

MATERIALS AND METHODS

Chemicals and reagents

Keratinocyte serum-free culture medium, trypsin, Hanks balanced salt solution and gentamicin were obtained from Life Technologies. The luciferase assay system was from Promega. Dispace and Fugene-6 were obtained from Boehringer-Mannheim. Chemiluminescence was measured on a Berthod

Abbreviations used: CMV, cytomegalovirus; DRR, distal regulatory region; hINV, human involucrin.

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luminometer, and the ECL[®] enhanced chemiluminescence system was obtained from Amersham Life Science Technology.

Plasmid construction

The involucrin promoter/luciferase gene constructs pINV-2473, pINV(-2473/-2088) and pINV(-2140/-2088) have been described [16]. The empty expression vector pCMV (in which CMV stands for cytomegalovirus) and the Sp1 protein expression vector pCMV-Sp1 were a gift from Jon Horowitz (Duke Medical Center, Durham, NC, U.S.A.) [28]. pSP1-4BS is a promoterless plasmid that encodes four Sp1-binding sites (4BS) from the SV40 enhancer. To construct pSP1-4BS, a DNA segment encoding the four Sp1-binding sites from the SV40 enhancer was isolated from plasmid pGL2-Promoter by digestion with *NcoI* followed by Klenow blunting and digestion with *BglII*. The released fragment was then cloned into *SmaI/BamHI*-restricted pBKS(+).

pTK-Luc, which contains the thymidine kinase minimal promoter upstream of luciferase, was the gift of John Nilson (Case Western Reserve University, Cleveland, OH, U.S.A.). pINV(-2473/-2088) was digested with *BglII*, blunted with Klenow polymerase and digested with *KpnI*. The released fragment containing the DRR was subcloned in the forward orientation into TK-Luciferase that was digested with *NheI*, blunted by Klenow polymerase and digested with *KpnI*. This created plasmid pTK(-2473/-2088). The correct joining of DNA sequences was verified by restriction mapping and/or DNA sequencing.

Tissue culture, transfection and luciferase assay

Primary human keratinocytes were cultured from human foreskin samples as described previously [29]. 3T3 fibroblasts and HEK-293 human embryonic kidney cells, a gift from Dr. George Dubyak (Case Western Reserve University), were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) calf serum at 37 °C in an air/CO₂ (19:1) atmosphere, and split 1:10 when 90% confluent. For transfection, cells were plated in 35 mm dishes 24 h before transfection. Fugene-6 (3 µl) was added to 97 µl of medium (without serum) and incubated at 25 °C for 5 min. The mixture was then mixed with 2 µg of hINV test plasmid, or 1 µg of hINV test plasmid and 1 µg of pCMV-Sp1 or pCMV, left at 25 °C for 15 min, and then added directly to the cells in 2 ml of serum-containing culture medium. The cells were harvested 48 h after transfection. The transfected cells were washed twice with PBS, lysed in 140 µl of cell lysis reagent (Promega), and harvested by scraping. Luciferase assays were performed as described previously [16].

Luciferase activity was normalized to parent luciferase reporter vector pGL2Basic, and expressed as fold activation relative to pGL2Basic. All transfections were done in duplicate and the fold activation represents the mean for three independent experiments. To ensure that cells were transfected at the same efficiency, 2 µg of pCMV-GFP, a plasmid that encodes green fluorescent protein, was transfected into each cell line in a separate set of plates. By using a fluorescence microscope (475 nm excitation peak, 490 nm emission peak) to monitor the production of green fluorescent protein, we were then able to compare the total cell number and the transfected cell number for each experiment. Transfection efficiencies were closely similar (routinely 70%) in all cell lines tested and did not vary within a given experiment.

Immunoblot methods

For immunoblots, cells were lysed in sample buffer [62.5 mM Tris/HCl (pH 6.8)/10% (v/v) glycerol/1.5% (v/v) SDS/4%

(v/v) 2-mercaptoethanol/0.001% Bromophenol Blue] at 250 pl per cell and boiled for 90 s; 8 × 10⁴ cell equivalents per sample were separated on an 8.5% (w/v) polyacrylamide gel. The separated proteins were transferred to nitrocellulose by electroblotting at 100 V for 1 h at 4 °C and the blot was incubated overnight with an Sp1-specific primary antibody (SC-59X; Santa Cruz Biotechnology) at room temperature at a 1:2500 dilution. hINV was detected by using a rabbit anti-hINV antibody diluted at 1:5000 [30]. Antigen-antibody complexes were detected with the ECL[®] system (Amersham Life Science Technology).

RESULTS

hINV promoter and DRR

pINV-2473 contains the full-length involucrin promoter fused to luciferase (Figure 1) [15,16]. pINV(-2473/-2088) encodes the DRR, which contains both strong (-2140/-2088) and weak activator (-2473/-2216) elements [16]. pINV(-2140/-2088) encodes only the strong activator element of the DRR linked to the hINV basal promoter [16].

Cell-type-specific expression of hINV promoter constructs

In vivo, involucrin is expressed only in stratifying squamous epithelial cells [7]. 3T3 fibroblast and HEK-293 cells represent cells of non-epithelial origin. The constructs shown in Figure 1 were transfected into cultured human keratinocytes, 3T3 fibroblasts and HEK-293 cells, and assayed for their ability to drive the production of luciferase (Figure 2). pINV(-2473/-2088) and pINV(-2140/-2088) produced high-level transcriptional activity in human keratinocytes (i.e. respectively 9-fold and 4-fold the amounts of the full-length hINV promoter), which is consistent with our previous results [16]. In contrast, the activity of pINV-2473, pINV(-2473/-2088) and pINV(-2140/-2088) was at near-basal levels in 3T3 and HEK-293 cells. These results suggest that the DRR segment might have an important role in specifying cell-type-specific expression.

We know from our previous study in keratinocytes that the weak activator element (-2473/-2140) doubles the activity of the strong activator element (-2140/-2088) [16]. Although promoter activity is very low in 3T3 and HEK-293 cells, addition of the weak activator element to the strong activator element produced a 2-fold (3T3) and 3-fold (HEK-293) increase in promoter activity. This implies that the strong activator element has a cell-type specific role, whereas the weak activator element serves to modulate activity, regardless of cell type.

Sp1 levels in keratinocytes and non-keratinocytes

Previous studies suggest that Sp1, a ubiquitously expressed general transcription factor, can participate in mediating the cell-specific expression of epidermal genes [24-26]. Thus the low activity of the hINV promoter constructs in 3T3 and HEK-293 cells could have been due to a difference in level of Sp1 protein. As shown in Figure 3, Sp1 levels in 3T3 (lane 2) and HEK-293 (lane 4) cells, as measured by immunoblotting, are one-fifth to one-tenth those in keratinocytes (lane 1), consistent with an effect of Sp1 concentration on expression.

Sp1 transfection can activate hINV expression

Sp1 levels are known to vary more than 100-fold in different tissues [31], and a previous study shows that keratinocytes

Figure 1 hINV promoter reporter plasmids

pINV -2473 encodes the full-length hINV promoter [16]. The five AP1-binding sites (●) and the single Sp1-binding site (■) are indicated. pINV(-2473/-2088) encodes the DRR, nt -2473/-2088, which encodes the weak and strong activator elements [16]. pINV(-2140/-2088) encodes the strong activator element which contains the AP1-5-binding site (box) and the Sp1-binding sites (cylinder), separated by a single nucleotide. Nucleotides are numbered relative to the transcription start site (arrow) [16]. The rightward arrow indicates the TATA box-containing hINV basal promoter (nt -41/-7).

Figure 2 Cell-type-specific expression of hINV promoter constructs

The plasmids shown in Figure 1 (2 μ g) were transfected into normal human keratinocytes, 3T3 fibroblasts and HEK-293 cells. Luciferase activity was measured as described in the Materials and methods section. The results are expressed as fold activation relative to pGL2-Basic, the parent plasmid for the involucrin reporter constructs. The transfection efficiency, as determined by visual inspection of GFP expression plasmid-transfected cells, was closely similar for all cell lines. The results are representative of three independent experiments. The difference between values within each group was 5% or less in this experiment and in the experiments shown in Figures 4, 5 and 6.

express Sp1 at a high level [27]. To evaluate whether increased levels of Sp1 in 3T3 and HEK-293 cells would increase hINV promoter activity, we co-transfected hINV reporter constructs with Sp1 expression vector or empty control vector. As shown in Figure 4 (upper panel), transfection of Sp1 activated the expression of all three hINV promoter constructs in 3T3 cells. A similar activation was observed in HEK-293 cells (Figure 4, lower panel). Co-transfection with Sp1 also doubled the promoter activity in keratinocytes (results not shown). As shown by immunoblotting in Figure 3, the activation of hINV promoter activity by Sp1 co-transfection was correlated with increased

Figure 3 Intracellular Sp1 levels

Total cell lysates were prepared from keratinocytes (KER), 3T3 cells and HEK-293 cells transfected with 1 μ g of empty expression vector, and from 3T3 and HEK-293 cells (3T3 + Sp1 and HEK + Sp1) transfected with 1 μ g of pCMV-Sp1. Equivalent quantities of extract (8×10^4 cell equivalents) were subjected to electrophoresis on an 8.5% (w/v) polyacrylamide gel; separated proteins were transferred to nitrocellulose and incubated with an Sp1-specific antibody. Binding of the primary antibody was detected as described in the Materials and methods section. The arrow indicates the position of the 95 and 105 kDa forms of Sp1 protein. The positions of markers are indicated (in kDa) at the left.

intracellular Sp1 levels. The level of Sp1 in transfected 3T3 (Figure 3, lane 3) and HEK-293 cells (lane 5) was greater than that observed in keratinocytes. Thus Sp1 can activate hINV promoter activity in cells that normally do not express hINV, and Sp1 can increase expression in keratinocytes.

Sp1 consensus binding site oligomer inhibits activity

The above studies demonstrate that co-transfected Sp1 can activate transcription of the hINV promoter. However, as shown in Figure 3, transfection studies inevitably lead to the high-level expression of transfected proteins in cells. To obtain additional evidence of a physiological role for Sp1 in the regulation of hINV in keratinocytes, we co-transfected pINV-2473 with a promoterless plasmid, pSP1-4BS, containing four Sp1 consensus binding sites from the SV40 promoter. Theoretically the Sp1-site plasmid should titrate the endogenous Sp1 protein away from the hINV promoter and result in decreased promoter activity. We had previously used this approach to study the regulation of

Figure 4 Sp1 co-transfection activates the hINV promoter in 3T3 and HEK-293 cells

Upper panel: 1 μ g of hINV promoter construct was co-transfected with 1 μ g of pCMV (KER) or with 1 μ g of pCMV-Sp1. A parallel experiment for HEK-293 cells is shown in the lower panel. The results are expressed as fold activation relative to the activity of pGL2-Basic. It is important to note that the fold hINV promoter activation observed in keratinocytes in this experiment is less than that described in Figure 2. This is due to a suppressive effect of the pCMV plasmid on hINV promoter activity.

promoter activity by POU domain factor [32]. To test this, 1 μ g of pINV-2473 was co-transfected with 0–1000 ng of pSP1-4BS. The total amount of plasmid in each transfection was maintained at 2 μ g by the addition of pBKS(+), the parent vector of pSP1-4BS. As shown in Figure 5, increasing concentrations of pSP1-4BS produced a progressive decrease in promoter activity.

Evidence that the cell-type-specific element is localized in the DRR element and not in the hINV basal promoter

The above experiments suggested that the DRR is activated in a cell-type-specific manner; however, the DRR constructs tested all contained the hINV basal promoter (–41/–7), which includes the TATA box. Therefore cell-type-specific regulation might depend on an interaction between the DRR and the hINV basal promoter or could reside exclusively in the basal promoter. To exclude a contribution of the hINV TATA box, we subcloned the DRR upstream of a thymidine kinase minimal promoter linked to luciferase. As shown in Figure 6, pTK(–2473/–2088) was expressed at a high level in keratinocytes and at near basal levels in 3T3 and HEK-293 cells, demonstrating that the

Figure 5 Promoter activity is decreased by reducing effective Sp1 concentration

Keratinocytes were transfected with 1 μ g of pINV-2473 and various concentrations of pSP1-4BS (+ SP1-4BS). Transfections included 0 (C), 10, 100 and 1000 ng of pSP1-4BS plasmid. In each case the total plasmid concentration was adjusted to 2 μ g by addition of pBKS(+), the parent vector for pSP1-4BS. The results are expressed as fold activation relative to the activity of pGL2-Basic. The dotted line indicates the activity in the presence of pBKS(+) with no pSP1-4BS.

specificity of expression lies in the DRR region and not in the TATA box. To ascertain whether the DRR, upstream of a heterologous promoter, is still responsive to Sp1 in cells of non-epithelial origin, 1 μ g of pTK(–2473/–2088) was transfected with 1 μ g of pCMV or 1 μ g of pCMV-Sp1 (Figure 6). The activity of pTK(–2473/–2088) in 3T3 and HEK-293 was increased to 50% of the activity present in keratinocytes. This result exactly paralleled that shown in Figure 4, in which the DRR was linked to the hINV basal promoter.

Sp1 expression activates the endogenous hINV gene in HEK-293 cells

Figure 3 shows that Sp1 levels are low in 3T3 and HEK-293 cells and can be markedly increased by transfection with Sp1 ex-

Figure 6 DRR drives cell-type-specific expression when linked to the thymidine kinase TATA box

3T3 and HEK-293 cells were transfected with 1 μ g of pTK(–2473/–2088) and 1 μ g of pCMV (–) or pCMV-Sp1 (+). Activity was compared with that observed in untransfected keratinocytes (KER). The results are expressed as fold activation relative to pTK-LUC.

Figure 7 Sp1 activates endogenous hINV gene expression in HEK-293 cells

Total cell lysates were prepared from keratinocytes (KER), 3T3 cells and HEK-293 cells transfected with empty vector or Sp1 expression vector as described in the legend to Figure 3. The transfection efficiency was 70%. Equivalent quantities of extract (8×10^4 cell equivalents) were subjected to electrophoresis on an 8.5% (w/v) polyacrylamide gel; separated proteins were transferred to nitrocellulose and incubated with an hINV-specific antibody. The arrow indicates the position of the hINV protein. The positions of markers are indicated (in kDa) at the left.

pression vector (Figure 3). In the experiment shown in Figure 7 we determined the effect of increased Sp1 levels on the expression of the endogenous hINV gene in 3T3 and HEK-293 cells. hINV protein was detected in HEK-293 cells transfected with Sp1 expression vector (Figure 7, lane 5), but not in cells transfected with empty expression vector (lane 4). No hINV protein was detected in control or Sp1 expression vector-transfected 3T3 cells (Figure 7, lanes 3 and 4 respectively). The protein detected in Sp1 expression vector-transfected HEK-293 cells co-migrated with the hINV present in keratinocyte cell extracts (Figure 7, lane 1).

DISCUSSION

Cell-specific activity of hINV promoter and DRR

In vivo, hINV protein is expressed only in stratifying squamous epithelial cells [33]. Studies on transgenic mice confirm that the full-length promoter is sufficient to drive tissue- and differentiation-specific expression [14]. However, the mechanism whereby involucrin is regulated in an epithelial cell-type-specific, differentiation-dependent fashion remains poorly understood. In a previous study we reported that the DRR of the hINV promoter contains elements that drive transcription in cultured keratinocytes [16]. We speculated that this region might have a role in the cell-type-specific expression of hINV. The DRR includes weak and strong activator elements (Figure 1) [16], and in keratinocytes the weak activator element functions to enhance the activity of the strong activator element [16]. The results with 3T3 and HEK-293 cells suggest that the weak element also functions as a general activator in non-epithelial cell types. This suggests that the strong activator element, which contains AP1-(AP1-5) and Sp1-binding sites separated by a single nucleotide, is a cell-type-specific enhancer and might participate in cell-type-specific regulation.

Sp1 as a regulator of cell-type-specific expression

Sp1 is a ubiquitously expressed, zinc finger-containing, sequence-specific DNA-binding protein [34,35]. At first sight it seems contradictory that a ubiquitously expressed general transcription factor such as Sp1 could mediate tissue-specific expression. However, keratins K5, K7, K4, K14 and K18, E-cadherin and SPRA2 are expressed in an epithelium-specific fashion and, in each case, the regulation involves ubiquitously expressed tran-

scription factors [17–23,36]. Sp1 protein level is known to vary 100-fold in different cell types [31] and is known to change during keratinocyte differentiation. In keratinocytes, for example, Sp1 levels are up-regulated as cells differentiate [27]. When Sp1 protein levels are compared in keratinocytes, 3T3 and HEK-293 cells by immunoblotting, keratinocytes express significantly more Sp1 than 3T3 and HEK-293 cells. The hINV promoter is barely detectable in 3T3 and HEK-293 cells; increasing the Sp1 level, by transfection of Sp1, activates hINV expression. Thus regulation of the Sp1 level is one possible mechanism whereby hINV promoter activity can be controlled. Sp1 is reported to regulate the expression of other genes expressed in stratifying squamous epithelia, including transglutaminase type 3, SPRA2 and keratins K3 and K5 [24–26,36].

Both the full-length hINV promoter and DRR constructs can be activated by Sp1 co-transfection in non-epithelial cells, providing the first example of hINV expression in a non-epithelial cell type. However, when expressed relative to the promoter activity in keratinocytes, the full-length promoter is more efficiently activated. This suggests that the full-length promoter contains elements, located outside the DRR, that are important for activation. This does not seem to be due to the presence of additional Sp1 sites because the DRR Sp1 site is the only Sp1 site present in the hINV promoter. It is possible that Sp1 functions by activating the production or modulating the activity of another transcription factor that activates via binding to a region outside the DRR. The PRR (proximal regulatory region) is a segment located adjacent to the TATA box that is transcriptionally active [15] and could mediate such a response.

The DRR AP1 site

The DRR also contains an AP1 site, AP1-5, one of five AP1 sites in the hINV promoter [15,16]. In the context of the DRR, mutation of this site results in a complete loss of promoter activity in all cell types [16], suggesting that AP1 does not function to drive cell-type-specific expression, but rather functions as a transcriptional 'on/off switch'.

Manipulating the endogenous Sp1 level alters hINV promoter activity

Sp1 co-transfection experiments provide evidence for a correlation between Sp1 level and promoter activity. Dose-response studies (results not shown) indicate that the activation of hINV promoter activity is directly related to the concentration of Sp1 expression plasmid. However, transfection can lead to the production of pharmacological levels of factor in cells. To confirm a role for endogenous Sp1 as an activator of hINV transcription in keratinocytes, we performed an Sp1-binding site titration experiment. We transfected keratinocytes with the hINV reporter construct in the presence of increasing concentrations of a plasmid containing multiple copies of a known Sp1-binding site. The binding sites sequestered Sp1 and decreased the amount of Sp1 available for interaction with the hINV promoter. This experiment, performed in keratinocytes, showed that when excess Sp1-binding sites are present, hINV reporter activity decreases by 65%, thus demonstrating that the hINV promoter is very sensitive to the ambient, endogenous, Sp1 level. *Drosophila* SL2 cells are widely used in Sp1 function studies, as they have been shown to be negative for Sp1 expression. hINV promoter constructs are completely inactive in SL2 cells, but promoter activity is increased 60-fold when the hINV promoter constructs are transfected with an Sp1 expression vector (results not shown). These results reinforce the Sp1-dependence of hINV promoter activity.

Sp1 activates the expression of the endogenous hINV gene

Involucrin is expressed only in stratifying surface epithelial cells [6,37]. Thus HEK-293 and 3T3 cells do not express hINV protein. However, our studies show that Sp1 can activate the hINV promoter in these cells. To provide additional evidence that Sp1 has a role in cell-type-specific regulation, we monitored the effects of Sp1 on the expression of the endogenous involucrin gene in HEK-293 and 3T3 cells. No involucrin is detected in 3T3 cells in the presence or absence of Sp1. This is expected, because 3T3 cells are derived from mouse tissue and the hINV-specific antibody does not cross-react with mouse involucrin [37,38]. Thus the experiment cannot be performed in 3T3 cells. However, transfection of Sp1 results in the production of hINV protein in HEK-293 cells. In comparison with the normal level of hINV gene expression in keratinocytes, the level of hINV protein detected in HEK-293 cells is modest. However, given the complexity of the transcriptional regulatory process (i.e. the possible role of DNA methylation and/or other transcriptional regulators), it is remarkable that Sp1 is able to produce any change in expression of the endogenous hINV gene. The fact that Sp1 expression can activate hINV gene expression in this cell line strongly supports the hypothesis that Sp1 is involved in cell-type-specific regulation.

Cell specificity of DRR is not mediated by the TATA box

The DRR constructs pINV(−2473/−2088) and pINV(−2140/−2088) were constructed by ligating the DRR region upstream of the hINV minimal promoter [16]; thus the cell-type-specific regulation of these constructs could be mediated via the hINV TATA box. However, this seems unlikely because when the DRR is subcloned upstream of a heterologous thymidine kinase minimal promoter, the DRR retains the same characteristics (i.e. cell-type-specific expression and Sp1 responsiveness) as when it is positioned upstream of the hINV TATA box. This suggests that the cell-type specificity is mediated via the DRR and not via the TATA box.

Possibility of cofactors

A change in Sp1 level is one way in which Sp1 could mediate cell-type-specific hINV expression; our results are consistent with this mechanism of Sp1 action. However, there might be other Sp1-dependent regulatory mechanisms. A large number of Sp1-interacting proteins have been identified, including GATA-1 [39], Ets-1 [40], Rb [28] and p53 [41]. In addition, Sp1 is known to interact with the general transcription machinery via TATA-binding-associated factor 110 [42]. Moreover, Sp1 is known to undergo post-translational phosphorylation and glycosylation [34], and Sp1 binding has been reported to increase 10-fold after dephosphorylation [43]. It is not known at present whether these alternative mechanisms are important for hINV gene regulation. In summary, our results support a role for Sp1 in regulating hINV gene expression. Our working hypothesis, as Sp1 is known to be expressed at high levels in differentiated keratinocytes [27,31], is that Sp1 protein acts to induce hINV expression in the suprabasal epidermal layers. Additional studies are needed to delineate further the role of Sp1.

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