VEGF mRNA expression in different stages of the human hair cycle: analysis by confocal laser microscopy

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Introduction

Hair growth during anagen is associated with a pronounced vascularisation of the hair bulb. In contrast to this, the vascular network is reabsorbed when the hair follicle enters into the catagen phase, and almost completely disappears in the telogen phase. Multiple growth factors or their receptors (e.g. bFGF, TGF-\(\beta_1,\) TGF-\(\beta_2,\) EGF ...) have been localized to the active follicle [1-3]. Since the expression of these factors changes during the hair cycle, they are implicated in the processes involved. Since vascular endothelial growth factor (VEGF) receptors are present in cultured dermal papilla cells of anagen hair follicles, and VEGF acts chemotactically and mitogenically on these cells [4], we hypothesise that VEGF may be involved in the initiation and/or development of the vascularization of the dermal papilla at the beginning of the anagen stage of the hair cycle. We suggest that VEGF secretion by the dermal papilla cells could support neovascularization of the hair bulb.

VEGF is involved in numerous vascular functions. It induces increased vascular permeability and tumour angiogenesis [5-7] and it is also a potent mitogenic agent for vascular endothelial cells [8-11]. VEGF is expressed by many types of normal [11] and tumour cells [5, 12, 13, 7]. Brown et al. [14] have shown that rat and guinea pig keratinocytes express VEGF mRNA during wound healing and secrete VEGF in primary culture. Others have characterized the different forms of VEGF produced by human keratinocytes in primary culture [15]. Elsewhere, VEGF has been examined for a pathogenic role in skin diseases characterized by neoangiogenesis and alterations of the epidermis. An overexpression of VEGF mRNA and its receptors flt-1 and KDR has been observed in psoriasis [16] and in epidermal lesions of patients affected by bullous pemphigoid, dermatitis herpetiformis, and erythema multiforme [17]. In all these diseases VEGF has been ascribed a role in the induction and maintenance of neovascularization. These factors led us to suppose that, during the hair cycle, VEGF may have a paracrine role in the induction of hair bulb vascularization and possibly a determinant role in hair growth. Therefore, we have investigated the expression of VEGF mRNA during different stages of the hair cycle using \textit{in situ} hybridization on scalp biopsies of normal subjects.
Materials and methods

In order to detect cells synthesizing VEGF-specific mRNA, we performed fluorescent in situ hybridization on frozen sections (5 μm) obtained from normal human scalp skin. The hybridization technique used was a modification of a procedure previously described. We used fluorescein labelled sense and antisense purified probes. The antisense probe hybridizes specifically with a region of VEGF mRNA common to all four VEGF splicing variants. Slides were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), rinsed twice in PBS, then incubated for 10 min in PBS, dehydrated in serial alcohols and air dried for 30 min. They were then overlaid with the hybridization buffer containing the antisense or sense probes (1 μg/ml). After mounting and sealing with rubber cement, the slides were incubated overnight. The sections were washed three times with Standard Sodium Citrate (SSC) 2x, blocked and incubated for 1h with anti-fluorescein diluted 1:100 in blocking solution. Finally, after several washings, the slides were mounted with a Vectashield mounting medium, before observation with a confocal laser scanning microscope (Zeiss). All slides were serially measured with the same voltage gain and sensitivity in any given series. Microscopic images were transferred to an image analysis dedicated work station (SUN IPC), where a specially developed software routine evaluated the number of fluorescent grains present in the follicle.

Results

As shown in Table 1, VEGF mRNA expression in the dermal papilla varied with the stage of the hair cycle, with the percentage of fluorescent spots being greatly reduced in catagen and telogen stages.

Table 1.
Quantification of VEGF transcripts in dermal papilla by confocal laser microscopy combined with image analysis.

<table>
<thead>
<tr>
<th></th>
<th>Number of spots</th>
<th>Spot surface area (μm²)</th>
<th>Derma papilla surface area</th>
<th>% Surface area Hybridized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anagen stage</td>
<td>2922</td>
<td>521</td>
<td>2900</td>
<td>18</td>
</tr>
<tr>
<td>Catagen stage</td>
<td>243</td>
<td>37</td>
<td>658</td>
<td>6</td>
</tr>
<tr>
<td>Telogen stage</td>
<td>175</td>
<td>34</td>
<td>854</td>
<td>4</td>
</tr>
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Dermal papillae (DP) from anagen follicles (Fig. 1B) revealed a considerable amount of VEGF mRNA, particularly at the base of the papillae. Weak fluorescence was seen in bulb matrix cells.
Figure 1. VEGF mRNA expression in anagen follicles. Bright-field (A) and corresponding dark-field (B) photomicrographs of in situ hybridization. Bar, 25 μm.

In catagen (Fig. 2B) and telogen stages (Fig. 3B), very, few or no grains of fluorescence were detected in dermal papillae, but the keratogenous zone was strongly positive. Controls which included sense transcripts were found to be negative. No specific labelling was seen with control sense probes in any hair follicle stage (data not shown).

Figure 2. VEGF mRNA expression in catagen. Bright-field (A) and corresponding dark-field (B) photomicrographs of in situ hybridization. Bar, 25 μm.

Figure 3. VEGF mRNA expression in telogen. Bright-field (A) and corresponding dark-field (B) photomicrographs of in situ hybridization. Bar, 25 μm.
Discussion

The formation of blood vessels in the hair bulb is of prime importance for hair growth. These processes involve the proliferation of endothelial cells and are likely to be regulated by angiogenic growth factors. We chose to study VEGF because it is the only known secreted mitogenic polypeptide factor that acts exclusively on vascular endothelial cells. In situ hybridization studies confirmed our immunohistochemical observations (unpublished data) with detection of VEGF in both dermal papillae and outer root sheath keratinocytes of anagen follicles. VEGF mRNA was strongly expressed in dermal papillae, particularly at the base. In contrast, dermal papillae from catagen and telogen follicles expressed little, or no, VEGF mRNA, while VEGF transcripts were highly expressed in matrix cells surrounding dermal papillae. This indicates that VEGF mRNA expression varies with the different hair cycle stages, with the greatest abundance being within the dermal papilla in anagen, more than in any other compartment of the hair follicle, and implies an important role of VEGF in the hair cycle. The reduced amounts of VEGF mRNA in dermal papillae during catagen and telogen are not surprising, because a low vascularization of the hair bulb is observed in these stages.

The high expression of VEGF in anagen dermal papillae may explain some of the characteristics of this richly vascularized follicle. This hypothesis is supported by the finding of Goldman et al. that VEGF is absent from hair follicles in alopecia areata and, to a lesser extent, in androgenetic alopecia. We suggest that the reduction of VEGF production may affect the maintenance of hair vascularization during the hair cycle. So that follicle cell function and, subsequently, hair growth may be regulated by VEGF.

In summary, the expression of VEGF mRNA only in dermal papilla at the anagen phase of the hair cycle appears to be linked to the induction function of the dermal papilla, which stimulates hair growth. It would be interesting to know if this pattern of expression is modified in dermal papilla from catagen and telogen stages and in all the compartments of hair follicle after treatment with different drugs.

References

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