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Expressional changes in the intracellular melanogenesis pathways and their possible role the pathogenesis of vitiligo

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Vitiligo;
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Summary

Background: Main pathway in human melanocytes through which signal from the melanocortin system reaches the melanogenesis enzymes is cAMP/PKA pathway and it is modulated by Wnt and MAPK pathways. In our previous study we established significant increase of melanocortin receptor expression in unaffected skin of vitiligo patients compared to healthy subjects.
Objective: The aim of this study was to assess the gene expression profile of the intracellular signalling pathways linking melanocortin system with enzymes involved in melanogenesis.
Methods: Using QRT-PCR method, mRNA expression levels of eight genes related to signal transduction from the melanocortin system to melanogenesis enzymes was measured in lesional and non-lesional skin of vitiligo patients and in the skin of healthy control subjects. Following genes were analyzed in the study: *MITF*, *CREB1*, *p38*, *USF1*, *PIK3CB* (*PI3K*), *RPS6KB1*, *LEF1* and *BCL2*.
Results: The mRNA levels of *MITF*, *LEF1*, *p38*, *PIK3CB* and *RPS6KB1* were decreased in lesional skin of vitiligo patients compared to skin of healthy control subjects. We also found increased expression of *USF1* and *BCL2* in non-lesional skin of vitiligo patients compared to skin of healthy control subjects. mRNA levels of *MITF* and *BCL2* were decreased in lesional skin of vitiligo patients compared to non-lesional skin of vitiligo patients.

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Conclusions: Present study indicates increased expression of the genes of the intracellular melanogenesis pathway in the non-lesional skin of vitiligo patients. This finding suggests activation of melanogenesis pathway in the non-lesional skin of vitiligo.

1. Introduction

Vitiligo is an acquired cutaneous disorder characterized by declining melanocyte functions and depigmentation. To explain the dysfunction of melanocytes in epidermis during the disorder different hypotheses have been proposed [1—3]. These hypotheses propose autoimmune mechanisms, auto-cytotoxic mechanism or abnormality in melanocytes or in surrounding keratinocytes leading to the decreased function of melanocytes [4,5]. However, as none of the three major hypothesis is sufficient to fully explain the mechanisms of vitiligo, the convergence theory is proposed stating that stress, accumulation of toxic compounds, infection, autoimmunity, mutations, altered cellular environment and impaired melanocyte migration and proliferation can all contribute to vitiligo etiopathogenesis in varying proportions [2].

In our previous study we showed significant alterations in the expression of genes of the melanocortin system in the skin of vitiligo patients [6]. We established reduced expression of melanocortin receptors and melanogenesis enzymes in lesional skin of vitiligo patients. Somewhat surprisingly we found that in non-lesional skin of the patients melanocortin receptors were significantly up-regulated. Increased expression of melanocortin receptors was accompanied by the up-regulation of the genes of enzymes involved in melanin synthesis [6,7]. As receptors and enzymes were altered to same direction, we concluded that this finding may possibly reflect the systemic compensatory changes to restore normal pigmentation in the lesions. To verify these findings further, we decided to analyze gene expression pattern in the intracellular signalling system linking melanocortin receptors with enzymes involved in melanin synthesis.

Different pathways modulate the melanogenesis in humans. Main pathway in human melanocytes through which signal from the melanocortin system reaches the melanogenesis enzymes tyrosinase (TYR), tyrosinase-related protein-1 (TYRP1) and dopachrome tautomerase (DCT) is cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway and it is modulated by Wnt and MAPK (mitogen-activated protein kinase) pathways. In addition, inositol phosphate/protein kinase C (IP3/PKC) and nitric oxide/protein kinase G (NO/PKG) pathways are able to modulate the melanin synthesis [3,8].

As cAMP/PKA is the most important pathway of pigmentation regulation, our study focused on this [9]. The melanocortin receptors activate adenylate cyclase (AC) leading to the rise of the intracellular cAMP concentration [10]. cAMP activates protein kinase A (PKA) [11]. PKA activates cAMP responsive element binding protein 1 (CREB1) through phosphorylation that raises the expression level of microphthalmia-associated transcription factor (MITF) [12]. MITF is a positive regulator of the expression levels of TYR, TYRP1 and DCT and increases the transcription of these enzymes [13—15]. MITF is the main gene responsible for the control of melanocyte differentiation; ectopic expression of MITF converts fibroblasts to cells with melanocyte characteristics [16]. In addition to other functions, MITF up-regulates the expression of the antiapoptotic factor B-cell lymphoma 2 (BCL2), the deletion of MITF in melanocytes results in an extensive apoptosis of these cells [17]. LEF1 (lymphoid enhancer-binding factor 1) is a transcription factor that participates in the Wnt signalling pathway [18]. In melanocytes, LEF1 acts as a pigmentation regulator, exerting its effects on MITF in two ways: firstly, LEF1 is an activator of the MITF gene transcription; secondly, MITF together with LEF1 can activate its own promoter [19]. The interaction of MITF and LEF1 also takes place in the regulation of the expression of DCT [20]. USF1 (upstream transcription factor 1) is a transcription factor that similarly with MITF belongs to the b-HLH-zip family [21]. In melanocytes, USF1 regulates pigmentation: when phosphorylated by 38 kDa MAP kinase (p38, ERK1/2), it binds to the promoter of TYR and activates its transcription [22]. As well, promoter of DCT contains the USF1 binding element [23]. Finally, cAMP has an inhibitory effect on the phosphoinositide 3-kinase/70 kDa ribosomal protein S6 kinase (PI3K/p70(S6)K) pathway [24].

Taken together, in order to evaluate potential changes in the intracellular signalling system, we analyzed the expression of the following genes: *MITF*, *CREB1*, *p38* (*ERK1*/2), *USF1*, *PIK3CB* (*PI3K*), *RPS6KB1*, *LEF1* and *BCL2*.