

Epidermal Expression of Melanocyte Related Proteins in Normal Skin and in Vitiligo

Sherif S. Awad, MD, PhD¹, Noha Moftah, MD¹, Sally Maher, MsC¹, Manal Gabril, MD, FRCPC (P) MSc^{2*}

¹Dermatology and Andrology department, Faculty of Medicine, Minia University, Egypt.

²Pathology and Laboratory Medicine, London Health Sciences Centre, Schulich School of Medicine & Dentistry, Western University, London, Canada.

*Corresponding Author: Sherif S. Awad, Dermatology and Andrology department, Faculty of Medicine, Minia University, Egypt.

Received date: March 26 2022; Accepted date: April 25, 2022; Published date: May 13, 2022

Citation: A Fabrice, A Hugues, D Juste, D Berenice, A Nadege, et al. (2022) Epidemiological profile of tumor dermatoses in the hospital setting in Cotonou (Benin) from 2009 to 2018. *Dermatology and Dermatitis*. 7(2); DOI:10.31579/2578-8949/099

Copyright: ©2022 Sherif S. Awad, this is an open-access article distributed under the terms of The Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Melanocytes are specialized neural crest-derived cells that are responsible for skin pigmentation. In vitiligo, loss of functioning epidermal melanocytes results in loss of pigment. Based on published studies, many transcription factors including microphthalmia-associated transcription factor, SRY (Sex Determining Region Y)-Box 10 are responsible for differentiation of melanocytes from neural crest cells as well as development of melanoblasts). However, the pathogenesis of loss of pigmentation in skin still unclear.

We studied the expression of several melanocyte-related proteins and markers in vitiligo samples and comparing them to normally pigmented skin samples. This may provide a more insight into the pathogenesis of vitiligo which is a still controversy.

We assessed the expression of six antibodies including S100, Melan-A (to detect fully formed, functioning melanocytes), CD117 (a specific relevant tyrosinase inducer), MITF and SOX-10 (important related transcription factors), and BCL-2 (stem cell markers) in 32 skin samples.

We demonstrated significant decline in the expression of all examined immunomarkers in the skin of vitiligo when compared to normal pigmented skin.

Our data may speculate that vitiligo pathogenesis does not involve only destruction of functioning active melanocytes, but also, other steps of cellular stimulation of induction of melanocytes and melanogenesis are significantly affected as well.

Keywords: vitiligo; immunomarkers; melanoblasts; melanocyte stem cells

Abbreviations

microphthalmia-associated transcription factor (MITF), SRY (Sex Determining Region Y)-Box 10 (SOX10), tyrosine kinase receptor KIT (c-kit), melanocyte stem cells (MelSCs), Dopachrome tautomerase gene (Dct),

Introduction

Melanocytes are specialized neural crest-derived cells that are responsible for skin pigmentation. The origin of melanocytes can be directly from neural crest cells migrating at the level of the skin through a dorsolateral migratory pathway, or from Schwann cell progenitors present in the peripheral nerves located at the level of the skin. Melanocytes reside in the basal layer of epidermis where they form the epidermal melanin units.

The ratio of melanocytes to keratinocytes is 1: 10 in the epidermal basal layer (Haass et al. 2005). Melanocytes produce melanin in melanosomes and transfer it to neighboring keratinocytes by using the dendrites, giving the skin color, and protecting the genetic material of keratinocytes from damage caused by UV radiation (Pisarchik A, Wortsman J 2004).

Melanoblasts are the melanocyte precursor cells and arise during gastrulation of embryogenesis at the dorsal edge of the neural crest (Thomas AJ 2009). The differentiation of melanocytes from neural crest cells as well as development of melanoblasts is controlled through complex molecular mechanisms mediated by a network of transcription factors, including microphthalmia-associated transcription factor (Mitf), SRY (Sex Determining Region Y)-Box 10 (SOX10), Paired Box 3 and Wnt proteins (Hou, Ling & Pavan 2008).

A key factor during melanocyte differentiation is microphthalmia associated transcription factor (Mitf) (Widlund HR 2003), (Hemesath, Timothy & Steingrímsson, Eiríkur & McGill, Gaél & J Hansen, M & Vaught, J & Hodgkinson 1994). MITF controls the expression of key pigment synthetic genes including TRP-1, dopachrome tautomerase and tyrosinase (Lin, Connie et al 2002). Various extracellular signaling pathways converge on MITF to control both migration and survival of melanoblasts (Tabone-Eglinger S., et al 2012).

Dependency on tyrosine kinase receptor KIT (c-kit) receptor and its ligand stem cell factor (SCF) is well known (Murphy, M., Reid, K. et al 1992), (Cable, J., et al 1995), (Sarin, Kavita & E Artandi 2007). In the bulge region of the hair follicle, melanoblasts can differentiate into melanocytes or can remain as melanocyte stem cells (MelSCs) (Kubic D, Jennifer, et al 2008).

Sox10 is also a neural crest transcription factor crucial for specification, maturation, and maintenance of Schwann cells and melanocytes. One of the studies suggested that SOX10 binding sites contribute significantly to the cAMP responsiveness of the Mitf promoter (Huber, W.E., et al 2003). Some of *in vitro* and *in vivo* tests showed that some melanogenic genes are stimulated not only by Mitf but also directly by SOX10, a regulator of MITF, suggesting the existence of feed-forward loops between SOX10 and Mitf target genes. Without functional MITF, however, melanocytes can never mature and become pigmented, but when they remain unpigmented because they have mutations in the melanin biosynthetic genes, they stay alive and thrive (Bauer GL, et al 2009).

Melanocytes molecularly are recognizable by identification of melanocyte-specific proteins as TYR, TYRP1, TYRP2, melanosomal matrix proteins as Pmel17, MART-1, and transcription factors such as microphthalmia transcription factor (MITF) (Plonka PM, et al 2009).

In vitiligo, loss of functioning epidermal melanocytes results in loss of pigment and induction of leukoderma characterized by disfiguring macules and patches of depigmented skin. (Arıcan O and Kurutas EB 2008).

The aim of this work is to monitor the expression of several melanocyte related proteins in normal skin and to compare that to the expression in vitiligo skin. This may provide a more insight into the pathogenesis of vitiligo which is a still controversy.

Results

3.1 Clinical data

The present study included 16 patients with vitiligo vulgaris. Eleven female patients (68.8%) and 5 males (31.2%) were included in this study. The age of these patients ranged from 12 to 54 years old (mean 21 ± 10.9). The disease duration ranged from 1 to 20 years (6.81 ± 5.7). A positive family history of vitiligo presented in 4 patients (25%). Associated autoimmune diseases presented in 2 patients (12.5%) (one patient had alopecia areata, the other patient had idiopathic thrombocytopenic purpura). As regard skin type, 3 patients (18.8%) were skin type III, 10 patients (62.4%) were skin type IV and 3 patients (18.8%) were skin type V (Table 1).

Baseline characteristics	The study group N = 16 patients
Age (years) Range Mean \pm SD	12 - 54 21 ± 10.9
Sex [No(%)] Male Female	5 (31.2%) 11 (68.8%)
Activity of the disease [No(%)] Active Stable	10 (62.5%) 6 (37.5%)
Duration of disease (years) Range Mean \pm SD	1 - 20 6.81 ± 5.7
Family history [No(%)] Positive Negative	4 (25%) 12 (75%)
Associated disease [No(%)] Positive Negative	2 (12.5%) 14 (87.5%)
Skin type [No(%)] III IV V	3 (18.8%) 10 (62.4%) 3 (18.8%)

Table 1: clinical demographic data of patients

3.2 Results of immunohistochemistry staining:

S100 expression:

Examining the epidermis of the stained skin samples revealed that S100 positively stained cells were seen within the epidermis and at the basal layer. Scattered Cells were more abundant in normal skin compared to

vitiligo skin which also contained several positively stained cells within the epidermis yet away from basal layer. S100 values in vitiliginous lesion ranged from 12 to 120 (mean 45.4 ± 34.23). In the non-lesional normally pigmented skin, it ranged from 45 to 144 (mean 85.6 ± 30.9) with statistically significant difference ($p = 0.001$), compared to vitiliginous lesion (Figure 1).

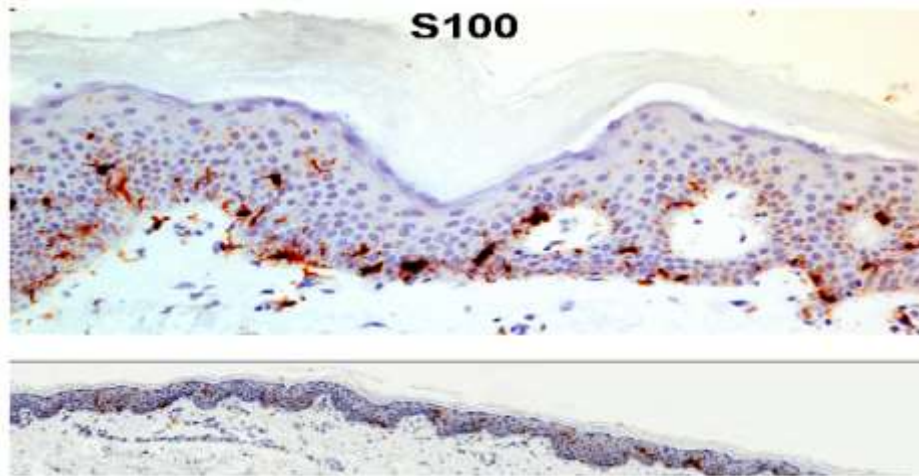


Figure 1: Expression of S100 in epidermis, Upper panel normal skin and lower panel vitiligo.

Melan-A expression:

Melan-A positively stained cells were demonstrated only within basal cells. Melan-A stained cells were minimal in vitiliginous lesion with

values ranged from 0 to 4 (0.25 ± 1). In the non-lesional normally pigmented skin, it ranged from 15 to 135 (71.4 ± 32.7) with statistically significant difference ($p = 0.001$) (Figure 2).

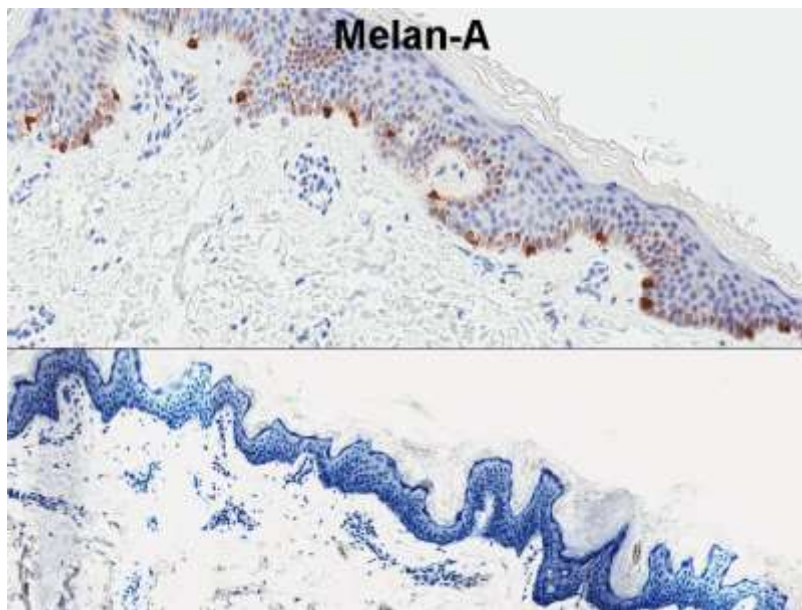


Figure 2: Expression of Mart-1 in epidermis, Upper panel normal skin and lower panel vitiligo.

CD-117 expression:

CD 117 (C-Kit) stained cells were demonstrated in the basal layer only. CD 117 stained cells in vitiliginous lesion were scanty and ranged from 0

to 10 (mean 1.12 ± 2.8). In the non-lesional normally pigmented skin, it ranged from 14 to 174 (mean 72.6 ± 52.3) with statistically significant difference ($p = 0.001$) (Figure 3).

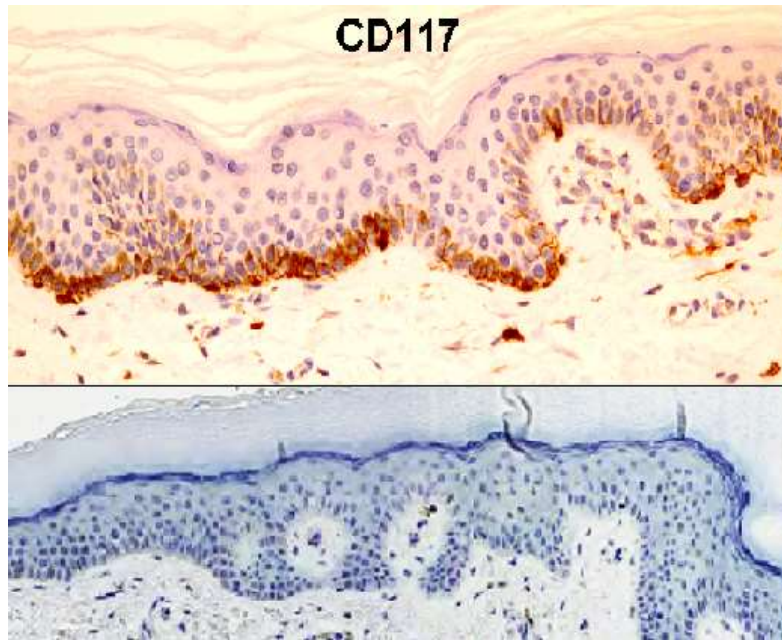


Figure 3: Expression of CD117 in epidermis, Upper panel normal skin and lower panel vitiligo.

MITF expression:

Mitf revealed positively stained cells within the basal layer. MITF stained cells showed values in vitiliginous lesion ranging from 0 to 12 (1.3 ± 3.4).

In the non-lesional normally pigmented skin, it ranged from 7 to 108 (73.7 ± 27.7) with statistically significant difference ($p = 0.001$) (Figure 4)

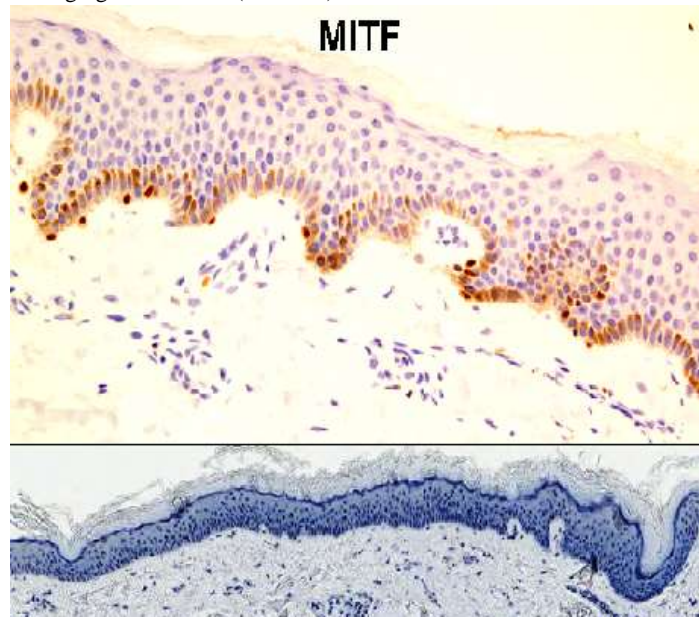


Figure 4: Expression of Mitf in epidermis, Upper panel normal skin and lower panel vitiligo.

SOX-10 expression:

SOX10 revealed positively stained cells within the basal layer. SOX10 stained cells in vitiliginous lesion were minimal with values ranged from

0 to 4 (0.25 ± 1). In the non-lesional normally pigmented skin, it ranged from 14 to 180 (64.1 ± 38.8) with statistically significant difference ($p = 0.001$) (Figure 5).

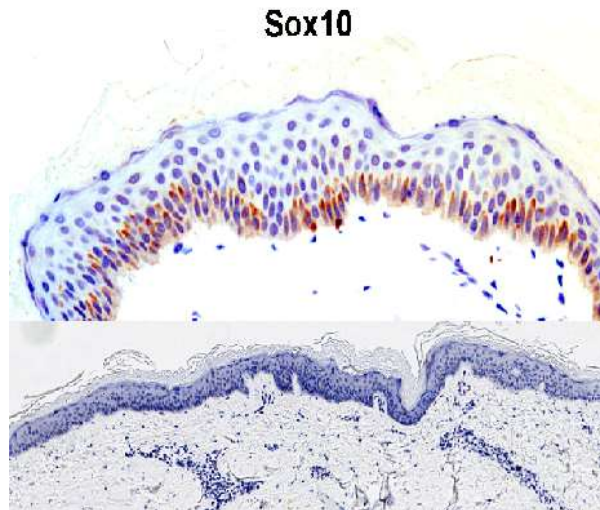


Figure 5: Expression of Sox10 in epidermis, Upper panel normal skin and lower panel vitiligo.

BCL-2 expression:

BCL-2 revealed positively stained cells within the basal layer. BCL-2 values in vitiliginous lesion ranged from 0 to 22 (7.1 ± 8.3). In the non-

lesional normally pigmented skin, it ranged from 10 to 180 (84.1 ± 50.1) with statistically significant difference ($p = 0.001$) (Figure 6).

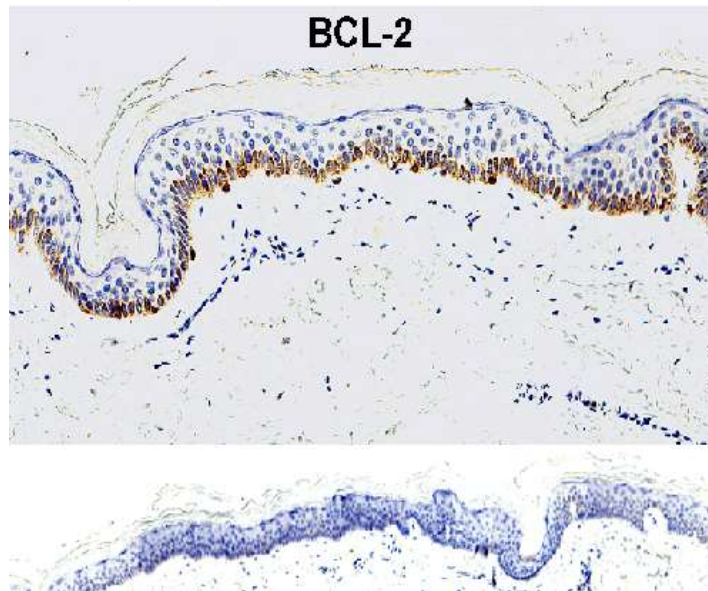


Figure 6: Expression of BCL2 in epidermis, Upper panel normal skin and lower panel vitiligo.

The overall expression values in vitiligo and normal skin are summarized in (Table 2 and Figure 7).

	Normal	Vitiligo
s100	85.6 ± 30.9	45.4 ± 34.23
Melan-A	71.4 ± 32.7	0.25 ± 1
CD117	72.6 ± 52.3	1.12 ± 2.8
Mitf	73.7 ± 27.7	1.3 ± 3.4

SOX10	64.1 ± 38.8	0.25 ± 1
BCL-2	84.1 ± 50.1	7.1 ± 8.3

Table (2): Comparison between epidermal expression values in normal and in vitiligo samples.

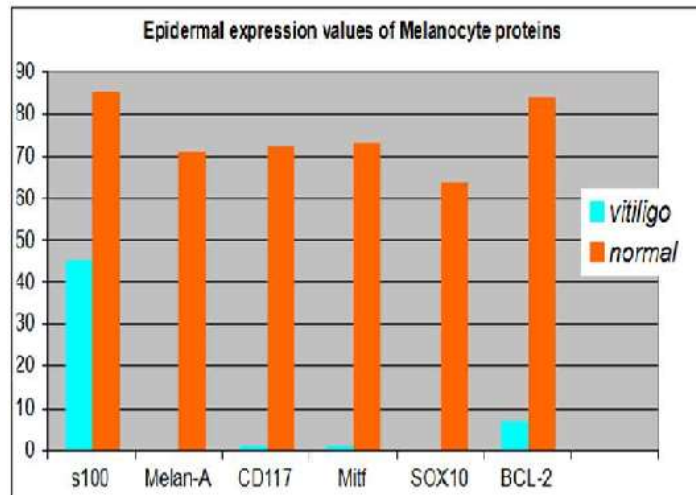


Figure 7: Graph showing the significant difference of expression of the studied proteins between normal skin and vitiligo.

Correlation of expression of all markers in epidermis of diseased skin specimens:

By comparing the expression of all markers in the epidermis of vitiliginous specimens, significant decline was demonstrated. However, there is a significant difference between S100 & BC12 in expression in vitiligo specimens, but none of the others.

Discussion

Melanocyte is a specialized cell which evolves from primitive skin stem cells and goes through a long and complicated process of differentiation, proliferation, migration, maturation and melanin synthesis. This study targeted the demonstration of expression of several melanocytes related proteins and markers in vitiligo samples and comparing them to normally pigmented skin.

S-100 and Melan A were used to detect fully formed and functioning melanocytes, CD117 was utilized as a specific relevant tyrosinase inducer, SOX10 and MITF were examined as important related transcription factors, and BCL-2 as stem cell markers.

It is known that, S100 protein can be expressed in different cells including fibroblasts, myoepithelial cells, histocytes and Langerhans cells in normal skin biopsies.(Xia J, et al 2016). We demonstrated that loss of S100 staining in vitiligo was mainly at basal layers as other non-melanocytic cells are still present intra-epidermally like Langerhans cells. Yet, significant drop in total values of expression in the epidermis was demonstrated due to the loss of functioning melanocytes located in the basal layer.

On the other hand, Melan-A expression is known to be restricted to melanin-producing cells, including normal and transformed skin melanocytes (Kawakami Y, et al 1994). Melan-A may be a melanosomal membrane protein, although its sequence does not bear any apparent homology to other known melanosomal proteins and does not possess

melanogenic enzymatic activities(Kawakami Y, et al 1997). Our study was clearly demonstrating that Melan-A expression significantly decreased in vitiligo skin compared to normal skin. This data is comparable to previously reported studies and minimal persistence of some positive cells is also recorded before (Mandelcorn-Monson RL, et al 2003) (Park OJ, et al 2016).

CD117 was also examined as it is a known important and specific inducer of melanocytes and melanogenesis. Significant drop in expression of this was clearly demonstrated in vitiligo. The intracellular signaling from the c-Kit plays a critical role in the development of melanocytes. Downstream signal transduction molecules can be activated through the formation of the c-kit/SCF complex, and then regulate gene expression and cell growth, proliferation and differentiation (Nishikawa S, et al 1991), (Grabbe J, et al 1994), (Galli SJ, et al 1995). There was a significant decline in C-kit expression in vitiligo compared to normal skin and this decline is similar to previous reports although was non-significant in many cases (Mandelcorn-Monson RL, et al 2003),(Park O, et al 2016).

Transcription factors and proteins are required during the process of melanocytes induction and formation from precursors. The microphthalmia-associated transcription factor (Mitf) protein is a key regulator of melanocyte development. The function of Mitf has been particularly well-studied in melanocytes, and it has been shown that Mitf is involved in the maintenance and self-renewal of melanocyte stem cells (Lang D1, et al 2005) (Loercher AE, et al 2005). Mitf can efficiently transactivate the melanogenesis enzyme genes, such as tyrosinase and tyrosinase-related protein 1 (TRP-1) in cultured cells (Yasumoto K, et al 1994), (Yasumoto K, et al 1997) ,(Amae S, et al 1998) ,(Shibahara S, et al 1999). A significant drop in expression of this factor was demonstrated in vitiligo samples compared to normal pigmented skin. Bhardwaj et al has been described similar findings in a recent study as well (Bhardwaj S, et al 2017).

SOX10 transcription factor belongs to the Sox-family of transcription factors important in the development and maintenance of melanocytes. It was significantly declined in vitiligo compared to expression in normal skin. In the melanocyte lineage, SOX10 directly regulates the expression of the *Mitf* gene (Bondurand N1, et al 2000), (Potterf SB, et al 2000), (Lee M, Goodall J, et al 2000) . and SOX10 also regulates the expression of the Dopachrome tautomerase gene (*Dct*), which functions in melanin biosynthesis (Britsch S, et al 2001) ,(Potterf SB, et al 2001) . It was demonstrated the elevation of expression of sox 10 in melanocytic tumors (Shin J, et al 2012) yet studying its expression in vitiligo is lacking and this could be first study to show its decline in vitiligo skin.

Stem cells are required to provide generations of melanocytes needed for the pigmentation process. No single marker is currently universally accepted as an identifier of all melanocyte stem cells (Bonchak JG, et al 2014) and here we examined for BCL-2. The proteins of the Bcl-2 family are important regulators of programmed cell death (Oltvai ZN, Milliman CL 1993) . BCL-2 interacts with *Mitf* to maintain survival of melanocyte (McGill GG, et al 2002). There is an indispensable role of BCL-2 in the development and maintenance of melanocyte stem cells (Hallsson JH, et al 2007) and so; mice null bcls lose pigment and melanoblasts decline on lose of BCL-2 (Mak SS, et al 2005). In our study BCL-2 was significantly decreased in the vitiliginous areas. Although some others, previously, denied any dysregulation of apoptosis molecules including BCL-2 in vitiligo (van den Wijngaar, et al 2000).

This study demonstrated the significant decline of several proteins and markers from the skin of vitiligo when compared to normal pigmented skin. It is comparable to many previous reports regarding MART-1, S100, and for a few reports regarding C-Kit expression or other transcription factors like *Mitf* yet it is the first report to signify the decline of SOX10, and BCL-2 in vitiligo skin.

In conclusion, Vitiligo skin lacks melanocytes and subsequently loses its melanocyte specific markers and proteins like Melan-A significantly. S100, although non-specific and can stain different types of cells within the epidermis, yet its expression in vitiligo also showed a significant drop compared to non lesional skin. The vital melanogenic factor C-Kit protein again demonstrated significant decline. Transcription factors and proteins required for induction of melanocyte and melanogenesis, *Mitf* and SOX10 were also significantly decreased. Stem cell related proteins and markers, BCL-2, significantly drop in vitiligo skin compared to non lesional skin and this may explain the persistent lack of the repigmentation in many cases and the characteristic recalcitrance of the disease.

Such findings may demonstrate that vitiligo pathogenesis does not involve only destruction of functioning active melanocytes, yet, other steps of early cellular stimulation of induction and melanocyte precursors are targeted and significantly affected as well.

This is a possible new view demonstrating that vitiligo could be a problem of homeostasis. In normal skin, not only functioning melanocyte proteins like Melan-A and CD117 exist but precursor and MelSCs related proteins like SOX-10, *Mitf*, BCL-2 also exist, so as to continually provide new generations of pigment producing cells to keep this pigmentary homeostasis. But in vitiligo, loss of those melanogenic machinery components leads to stoppage of production process from a very early step. Vitiligo skin cannot maintain the usual existence of melanocytes in the skin through continual stimulation of melanocyte precursor's lineage due to lack of required precursors and their relevant proteins.

2. Material and Methods

2.1 Clinical data

The study included 32 skin samples obtained using 4mm punch probes. Half of them were obtained from center of depigmented vitiligo skin of non-segmental vitiligo cases and the other half from normally pigmented skin away from the lesions. Biopsies were immediately fixed in 10% formalin, routinely processed and embedded in paraffin blocks.

2.2 Immunohistochemical staining and scoring:

Immunohistochemical staining was performed on the paraffin-embedded specimens using a semi-automated Discovery autostainer (Dako EnVision FLEX+ detection system) and EnVision FLEX+ DAB+ Chromogen.

Tissues were exposed to 7 different primary antibodies including: 1- S100: polyclonal rabbit anti-S100 (code no.: IR504, Dako, North America) is used. Melanocytic cells were scored positive if they showed cytoplasmic or nuclear staining. 2- Melan-A: FLEX monoclonal Mouse Anti-Human Melan-A (code IR633, Dako, North America) is used. Cells were scored positive if they showed cytoplasmic staining. 3- CD117: polyclonal Rabbit Anti-Human CD117 (c-kit) antibody (code no.: A4502, Dako, North America) at a dilution 1:100 is used. Cells were scored positive if they showed cytoplasmic staining. 4- MITF (microphthalmia transcription factor): monoclonal Mouse Anti-Human MITF antibody (code no.: M3621, Dako, North America) at a dilution of 1:100 is used. Cells were scored positive if they showed nuclear staining. 5- SOX10 monoclonal Mouse Antibody SOX10 (code: SC-365692, Santa Cruz Biotechnology, Europe) at a dilution of 1:300 is used. Cells were scored positive if they showed cytoplasmic or nuclear staining. 6- BCL-2: monoclonal Mouse Anti-Human BCL-2 Oncoprotein (code no.: IR614, Dako, North America) is used. Cells were scored positive if they showed cytoplasmic or nuclear staining.

2.3 Slides scanning:

The slides were examined and scanned by using Aperio glass slide scanner at Department of Pathology and laboratory Medicine at Western University. The ScanScope scans microscope slides at 20X, 40X to a digital image that can be viewed with software that simulates the use of a light microscope. The svf files can be viewed and manipulated using a free software program called ImageScope. Representative photographs were captured using Aperio ScanScope™ technology at equivalent exposure times.

2.4 Immunohistochemical scoring:

Evaluation of the epidermal expression was carried by counting the number of positively stained cells within the epidermis in 1 mm length of stained tissue in the obtained digital slide-photos and multiplying the number by the intensity of positive staining; 1 for mild staining, 2 for moderate intensity and 3 for strong. Three independent doctors carried out the evaluation and median value for each case was recorded for further statistical evaluation.

2.5 Statistical analysis

Clinical data were compared across the two groups using analysis of variance for differences in clinical parameters including age, sex, skin type, duration and activity of the disease and family history. A two-way repeated measures ANOVA with an interaction test and pairwise differences were used for comparing parametric quantitative data of two groups regarding the differences in the expression of six immunomarkers. All tests were two-sided, with a P-value of <0.05 considered to be significant.

We have no conflict of interest

References

1. Amae S, Fuse N, Yasumoto K, Sato S, Yajima I, Yamamoto H, et al. (1998). "Identification of a Novel Isoform of Microphthalmia-Associated Transcription Factor That Is Enriched in Retinal Pigment Epithelium." *Biochem Biophys Res Commun.* 247(3):710-715.
2. Amoh Y, Hoffman RM. (2017). "Hair Follicle-Associated-Pluripotent (HAP) Stem Cells." *Cell Cycle.* 16(22):2169-2175.
3. Arican O and Kurutas EB. (2008). "Oxidative Stress in the Blood of Patients with Active Localized Vitiligo." *Acta. Dermatoven APA.* 17(1):12-16.
4. Bauer GL, Praetorius C, Bergsteinsdóttir K, Hallsson JH, Grísladóttir BK, Schepsky A, et al. (2009). "The Role of MITF Phosphorylation Sites during Coat Color and Eye Development in Mice Analyzed by Bacterial Artificial Chromosome Transgene Rescue." *Genetics* 183(2): 581-594.
5. Bhardwaj S, Rani S, Srivastava N, Kumar R, Parsad D. (2017). "Increased Systemic and Epidermal Levels of IL-17A and IL-1 β Promotes Progression of Non-Segmental Vitiligo." *Cytokine.* 91:153-561.
6. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. (2004). "Self-Renewal, Multipotency, and the Existence of Two Cell Populations within an Epithelial Stem Cell Niche." *Cell* 118(5):635-648.
7. Bonchak JG, Eby JM, Willenborg KA, Chrobak D, Henning SW, Krzywiac A, et al. (2014). "Targeting Melanocyte and Melanoma Stem Cells by 8-Hydroxy-2-Dipropylaminotetralin." *Arch Biochem Biophys.* 563:71-78.
8. Bondurand N1, Pingault V, Goerich DE, Lemort N, Sock E, Le Caignec C, et al. (2000). "Interaction among SOX10, PAX3 and MITF, Three Genes Altered in Waardenburg Syndrome." *Hum Mol Genet.* 9(13):1907-1917.
9. Britsch S, Goerich DE, Riethmacher D, Peirano RI, Rossner M, Nave KA, Birchmeier C, et al. (2001). "The Transcription Factor Sox10 Is a Key Regulator of Peripheral Glial Development." *Genes Dev.* 15(1): 66-78.
10. Cable, J., Jackson, I. J., & Steel, K. P. (1995). Mutations at the Wlocus Affect Survival of Neural Crest-Derived Melanocytes in the Mouse." *Mechanisms of Development* 50:139-150.
11. Galli SJ, Tsai M, Wershil BK, Tam SY, Costa JJ. (1995). "Regulation of Mouse and Human Mast Cell Development, Survival and Function by Stem Cell Factor, the Ligand for the c-Kit Receptor." *Int Arch Allergy Immunol.* 107(1-3):51-53.
12. Grabbe J, Welker P, Dippel E, Czarnetzki BM. (1994). "Stem Cell Factor, a Novel Cutaneous Growth Factor for Mast Cells and Melanocytes." *Arch Dermatol Res.* 287(1):78-84.
13. Haass, Nikolas K., Keiran S. M. Smalley, Ling Li, and Meenhard Herlyn.(2005). "Adhesion, Migration and Communication in Melanocytes and Melanoma." *Pigment Cell Research Banner* 18(3):150-59.
14. Hallsson JH, Haflidadóttir BS, Schepsky A, Arnheiter H, Steingrímsson E. (2007). "Evolutionary Sequence Comparison of the Mitf Gene Reveals Novel Conserved Domains." *Pigment Cell Res.* 20(3):185-200.
15. Hemesath, Timothy & Steingrímsson, Eiríkur & McGill, Gaél & J Hansen, M & Vaught, J & Hodgkinson, Colin & et al. (1994). "Microphthalmia, a Critical Factor in Melanocyte Development, Defines a Discrete." *Genes & Development* 8 (22):2770-2780.
16. Hou, Ling & Pavan, William. (2008). "Transcriptional and Signaling Regulation in Neural Crest Stem Cell-Derived Melanocyte Development: Do All Roads Lead to MITF?." *Cell Research* 18(12):1163-1176.
17. Hsu YC, Pasolli HA, Fuchs E. (2011). "Dynamics between Stem Cells, Niche, and Progeny in the Hair Follicle." *Cell* 144 (1):92-105.
18. Huber, W.E. Price, E.R. Widlund, H.R. Du, J. Davis, I.J. Wegner, and et al M. (2003). "A Tissue-Restricted cAMP Transcriptional Response: SOX10 Modulates Alpha-Melanocyte-Stimulating Hormone-Triggered Expression of Microphthalmia-Associated Transcription Factor in Melanocytes." *J. Biol. Chem.* 278:45224-45230.
19. Kawakami Y, Battles JK, Kobayashi T, Ennis W, Wang X, Tupesis JP, et al. (1997). "Production of Recombinant MART-1 Proteins and Specific antiMART-1 Polyclonal and Monoclonal Antibodies: Use in the Characterization of the Human Melanoma Antigen MART-1." *J Immunol Methods.* 202(1):13-25.
20. Kawakami Y, Eliyahu S, Sakaguchi K, Appella E, Rosenberg S A et al. (1994). "Identification of the Immunodominant Peptides of the MART-1 Human Melanoma Antigen Recognized by the Majority of HLA-A2-Restricted Tumor Infiltrating Lymphocytes." *JEM* 180(1): 347.
21. Kinsler VA, Anderson G, Latimer B, Natarajan D, Healy E, Moore GE, et al. (2013). "Immunohistochemical and Ultrastructural Features of Congenital Melanocytic Naevus Cells Support a Stem-Cell Phenotype." *Br J Dermatol.*169(2):374-383.
22. Kubic D, Jennifer, Young P, Kacey, Plummer S, Rebecca & E Ludvik, Anton & Lang, Deborah. (2008). "Pigmentation PAX-Ways: The Role of Pax3 in Melanogenesis, Melanocyte Stem Cell Maintenance, and Disease." *Pigment Cell & Melanoma Research.* 21(6):627-645.
23. Lang D, Lu MM, Huang L, Engleka KA, Zhang M, Chu EY, et al. (2005). "Pax3 Functions at a Nodal Point in Melanocyte Stem Cell Differentiation." *Nature* 433 (7028): 882-887.
24. Lee M, Goodall J, Verastegui C, Ballotti R, Goding CR. (2000). "Direct Regulation of the Microphthalmia Promoter by Sox10 Links Waardenburg-Shah Syndrome (WS4)-Associated Hypopigmentation and Deafness to WS2." *J Biol Chem.*275(48): 37978-37983.
25. Lin, Connie & Babiarz, L & Liebel, F & Roydon Price, E & Kizoulis, M & Gendimenico, Gerry et al. (2002). "Modulation of Microphthalmia-Associated Transcription Factor Gene Expression Alters Skin Pigmentation." *The Journal of Investigative Dermatology* 119(6):1330-1340.
26. Lin CS, Ning H, Lin G, Lue TF. (2012). "Is CD34 Truly a Negative Marker for Mesenchymal Stromal Cells?" *Cytotherapy.* 14(10):1159-1163.
27. Loercher AE, Tank EM, Delston RB, Harbour JW. (2005). "MITF Links Differentiation with Cell Cycle Arrest in Melanocytes by Transcriptional Activation of INK4A." *J Cell Biol.* 168 (1):35-40.
28. Mak SS, Moriyama M, Nishioka E, Osawa M, Nishikawa S. (2005). "Indispensable Role of Bcl2 in the Development of the Melanocyte Stem Cell." *Developmental Biology.* 291:144-153.
29. Mandelcorn-Monson RL, Shear NH, Yau E, Sambhara S, Barber BH, Spaner D, DeBenedette MA. (2003). "Cytotoxic T Lymphocyte Reactivity to gp100, MelanA/MART-1, and Tyrosinase, in HLA-A2-Positive Vitiligo Patients." *J Invest Dermatol.* 121 (3):550-556.
30. McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, et al. (2002). "Bcl2 Regulation by the Melanocyte Master Regulator Mitf Modulates Lineage Survival and Melanoma Cell Viability." *Cell* 109(6):707-718.
31. Murphy, M., Reid, K., Williams, D. E., Lyman, S. D., & Bartlett, P. F. (1992). "Steel Factor Is Required for Maintenance, but Not Differentiation, of Melanocyte Precursors in the Neural Crest." *Developments in Biologicals*153:396-401.
32. Nakayama H, Enzan H, Miyazaki E, Kuroda N, Naruse K, Hiroi

- M. (2000). "Differential Expression of CD34 in Normal Colorectal Tissue, Peritumoral Inflammatory Tissue, and Tumour Stroma." *J Clin Pathol.* 53(8):626-629.
33. Nielsen JS, McNagny KM. (2008). "Novel Functions of the CD34 Family." *J Cell Sci.* 121: 3683-3692.
34. Nishikawa S, Kusakabe M, Yoshinaga K, Ogawa M, Hayashi S, Kunisada T, et al. (1991). "In Utero Manipulation of Coat Color Formation by a Monoclonal Anti-c-Kit Antibody: Two Distinct Waves of c-Kit-Dependency during Melanocyte Development." *EMBO J.* 10(8):2111-2118.
35. Oltvai ZN, Milliman CL, Korsmeyer SJ. (1993). "Bcl-2 Heterodimerizes in Vivo with a Conserved Homolog, Bax, That Accelerates Programmed Cell Death." *Cell* 74 (4): 609-619.
36. Park OJ, Han JS, Lee SH, Park CS, Won CH, Lee MW, et al. (2016). "Expression of Epidermal c-Kit+ of Vitiligo Lesions Is Related to Responses to Excimer Laser." *Ann Dermatol.* 28(4):457-463.
37. Pisarchik A, Wortsman J, Slominski A. (2004). "A Novel Microarray to Evaluate Stress-Related Genes in Skin: Effect of Ultraviolet Light Radiation." *Gene* 341:199-207.
38. Plonka PM, Passeron T, Brenner M, Tobin DJ, Shibahara S, Thomas A, et al. (2009). "What Are Melanocytes Really Doing All Day Long...?" *Exp Dermatol.* 18 (9): 799-819.
39. Potterf SB, Furumura M, Dunn KJ, Arnheiter H, Pavan WJ. (2000). "Transcription Factor Hierarchy in Waardenburg Syndrome: Regulation of MITF Expression by SOX10 and PAX3." *Hum Genet.* 107(1):1-6.
40. Potterf SB, Mollaaghababa R, Hou L, Southard-Smith EM, Hornyak TJ, Arnheiter H, et al. (2001). "Analysis of SOX10 Function in Neural Crest-Derived Melanocyte Development: SOX10-Dependent Transcriptional Control of Dopachrome Tautomerase." *Dev Biol.* 237(2):245-257.
41. Sarin, Kavita & E Artandi, Steven. (2007). "Aging, Graying and Loss of Melanocyte Stem Cells." *Stem Cell Reviews.* 3(3):212-217.
42. Shibahara S, Yasumoto K, Amae S, Fuse N, Uono T, Takahashi K. (1999). "Implications of Isoform Multiplicity of Microphthalmia-Associated Transcription Factor in the Pathogenesis of Auditory-Pigmentary Syndromes." *J Investig Dermatol Symp Proc.* 4(2):101-104.
43. Shin J, Vincent JG, Cuda JD, Xu H, Kang S, Kim J, et al. (2012). "Sox10 Is Expressed in Primary Melanocytic Neoplasms of Various Histologies but Not in Fibrohistiocytic Proliferations and Histiocytoses." *JAAD Case Reports* 67 (4): 717-726.
44. Tabone-Eglinger S, Wehrle-Haller M, Aebischer N, Jacquier M-C, and Wehrle-Haller B. (2012). "Membrane-Bound Kit Ligand Regulates Melanocyte Adhesion and Survival, Providing Physical Interaction with an Intraepithelial Niche." *FASEB J.* 26: 3738-3753.
45. Thomas AJ, Erickson CA. (2009). "The Making of a Melanocyte: The Specification of Melanoblasts from the Neural Crest." *Pigment Cell Melanoma Res.* 21:598-610.
46. Tumber T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, et al. (2004). "Defining the Epithelial Stem Cell Niche in Skin." *Science.* 303(5656):359-363.
47. Widlund HR, Fisher DE. (2003). "Microphthalmia-Associated Transcription Factor: A Critical Regulator of Pigment Cell Development and Survival." *Oncogene.* 22(20): 3035-3041.
48. Wijngaard RM, Aten J, Scheepmaker A, Le Poole IC, Tigges AJ, Westerhof W, Das PK. van den. (2000). "Expression and Modulation of Apoptosis Regulatory Molecules in Human Melanocytes: Significance in Vitiligo." *Br J Dermatol.* 143(3): 573-581.
49. Xia J, Wang Y, Li F, Wang J, Mu Y, Mei X, et al. (2016). "Expression of Microphthalmia Transcription Factor, S100 Protein, and HMB-45 in Malignant Melanoma and Pigmented Nevi." *Biomed Rep.* 5(3):327-331.
50. Yasumoto K, Yokoyama K, Shibata K, Tomita Y, Shibahara S. (1994). "Microphthalmia-Associated Transcription Factor as a Regulator for Melanocyte-Specific Transcription of the Human Tyrosinase Gene." *Mol Cell Biol* 14 (12): 8058-8070.
51. Yasumoto K, Yokoyama K, Takahashi K, Tomita Y, Shibahara S. (1997). "Functional Analysis of Microphthalmia-Associated Transcription Factor in Pigment Cell-Specific Transcription of the Human Tyrosinase Family Genes." *J Biol Chem.* 272(1): 503-509.



This work is licensed under Creative Commons Attribution 4.0 License

To Submit Your Article Click Here:

Submit Manuscript

DOI: [10.31579/2578-8949/099](https://doi.org/10.31579/2578-8949/099)

Ready to submit your research? Choose Auctores and benefit from:

- **fast, convenient online submission**
- **rigorous peer review by experienced research in your field**
- **rapid publication on acceptance**
- **authors retain copyrights**
- **unique DOI for all articles**
- **immediate, unrestricted online access**

At Auctores, research is always in progress.

Learn more <https://auctoresonline.org/journals/dermatology-and-dermatitis>