Immunohistochemical Study of Protein Gene Product 9.5 and Single Strand DNA in Generalized and Segmental Vitiligo

Thesis
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LIST OF ABBREVIATIONS

4-TBP: 4-tertiary butylphenol
5-MOP: 5-methoxypsoralen
8-MOP: 8-methoxypsoralen
AD: Anno Domini
ADCC: Antibody dependant cell mediated cytotoxicity
AISL: Autoimmune susceptibility locus
APC: Antigen presenting cell
Bax: B cell lymphoma-2 associated X protein
BC: Before Christ
BCL2: B cell lymphoma-2
bFGF: basic fibroblast growth factor
C: Complement
CAT: Catalase gene
CD: Cluster of differentiation
cDNA: Complementary DNA
CGRP: Calcitonin gene related peptide
CLA: Cutaneous lymphocyte associated antigen
COMT: Catechol-O-methyl transferase
CTLA: Cytotoxic lymphocyte antigen 4
DHICA: 5,6-Dihydroxyindole-2-carboxylic acid
EDTA: Ethylenediaminetetraacetic Acid
EGM: Extra cellular granular material
ET Endothelins
FGF: Fibroblast growth factor
H2O2: Hydrogen peroxide
H&E: Haematoxylin and Eosin
HCV: Hepatitis C virus
HIV: Human immune deficiency virus
HLA: Human leucocytic antigen
ICAM: Intracellular adhesion molecule
IDDM: Insulin dependent diabetes mellitus
IKP: Isomorphic Koebner phenomenon
IL: Interleukin
INF: Interferon
KDa: Kilo Dalton
KUVA: Khellin plus UVA
LAK: Lymphokine activated killer cell
LC: Langerhans’ cells
LSAB: Labeled StreptAvidin Biotin
MAO: Monoamino oxidase
MBEH: Monobenzyl ether of hydroquinone
MCHR: Melanin concentrating hormone receptor
MHC: Major histocompatibility complex
MITF: Microphthalmia-associated transcription factor
MSH: Melanocyte-stimulating hormone
NGF: Nerve growth factor
NK: Natural killer cell
NPY: Neuro peptide Y
PBS: Phosphate Buffered Saline
PGP 9.5: Protein gene product 9.5
ROS: Reactive oxygen species
SCF: Stem cell factor
ssDNA: Single stranded DNA
TAP1: Transporter associated with antigen-processing
TCR: T-cell receptor
TGFβ1: Transforming growth factor β
Th: T helper cell
TiO2: Titanium dioxide
TNF: Tumor necrosis factor
TRP: Tyrosinase related protein
VKHS: Vogt-Koyanagi-Harada syndrome
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A. INTRODUCTION

Vitiligo is an acquired dermatologic disorder characterized by loss of functioning melanocytes, resulting in depigmentation of the skin. (Tobin et al., 2000; Solano et al., 2006; Van Geel et al., 2014).

The mechanisms underlying the destruction of functioning melanocytes and the absence of melanin in vitiligo lesions remain unclear. Nevertheless, certain theories have been suggested and studied including; the genetic hypothesis, the autoimmune hypothesis, the neural hypothesis (involving neuropeptides, adrenergic and cholinergic neurotransmitters), the apoptotic theory, the viral hypothesis, the self destruction hypothesis (including the significant contribution of oxidative stress through the accumulation of H2O2), and convergence theory (which combines previous theories). (Cucchi et al., 2000; Dell’Anna et al., 2003; Gauthier et al., 2003; Ortonne, 2003; Hasse et al., 2004; Schallreuter et al., 2006; Solano et al., 2006).

Developmentally, melanoblasts are derived from the neural crest, and so it is not surprising that an association between neurological disorders and changes in skin pigmentation can often be found. The segmental distribution of vitiligo, and the association of vitiligo with peripheral nerve injury, viral encephalitis, horner’s syndrome and diabetic neuropathy, supports the neurological theory in vitiligo (Al’Abadie et al., 1994; Liu et al., 1999).

Protein gene product 9.5 (PGP 9.5) is a general marker for all cutaneous sensory and autonomic nerve fibers. It has been studied in skin biopsies of various dermatologic disorders (McArthur et al., 1998; Omdal et al., 2002; Antunes et al., 2003; Ebnezer and Daniel, 2004).

Studies of PGP 9.5 in vitiligo have been performed. One study showed a minimal increase in PGP 9.5 positive nerve fibers at the dermoepidermal junction and lower malpighian layers in patients with vitiligo at the periphery of the lesion relative to normal skin.
(Al’Abadie et al., 1994). Other reported no difference in PGP 9.5 positive nerve fibers between lesional, nonlesional, and normal skin in patients with vitiligo (Liu et al., 1999). However, recently Aroni et al., 2008 detected a statistically significant difference in the number of PGP 9.5-positive nerve fibers/axons in the papillary dermis between the centre and periphery of the lesions of vitiligo (i.e. increased at the center in comparison with the periphery).

A few controversial theories have been studied concerning the role of apoptosis in vitiligo. The lack of evidence for the involvement of this process has been reported in several studies (Tobin et al., 2000; Van den Wijngaard et al., 2000a). However vitiligo as a manifestation of apoptosis is supported by its histopathological findings, and is particularly evident from the changes at the border between the depigmented and clinically normal (uninvolved) skin (Kovarik et al., 2009).

A monoclonal immunoglobulin M (IgM) antibody was used by Aroni et al., 2008 against single strand DNA (ssDNA), which specifically stains the apoptotic cells and has been applied in vitiligo to differentiate between apoptotic and necrotic cells.

On the basis of dermal PGP 9.5-positive nerve fibers and ssDNA-positive (apoptotic) cells, Aroni et al., 2008 concluded that there is a relationship between the autonomic nerve system function and apoptosis, supporting the hypothesis that the destruction of functioning melanocytes in vitiligo could be the end result of different interacting pathogenic mechanism, such as apoptosis and accumulation of neural fibers/axons.
B. AIM OF THE WORK

The aim of this work is to study the possible contribution of either the neural mechanism or apoptotic mechanism or both together in the etiopathogenesis of generalized and segmental vitiligo variants. This was done through immunohistochemical study of PGP9.5 as evidence of neural mechanism and ssDNA as an evidence of apoptotic mechanism in vitiligo.