1.1 Vitiligo

Vitiligo is an acquired noncontagious, idiopathic, depigmentation disorder in which progressive loss of functional melanocytes result in patchy depigmentation of the skin (Le Poole and Boissy, 1997). The prevalence of vitiligo is reported to be around 0.2-1.8% worldwide, (Zhang *et al.,* 2016) and 0.5 to 2.5% in India based on a few dermatological outpatient records (Handa and Kaur 1999). Vitiligo usually starts in childhood or young adulthood; the clinical manifestation begins before 20 years of age in 50% of the cases, while in the rest 25% cases the onset is before the age of 14 years, without any gender biasness (Kakourou, 2009; Ezzedine *et al.,* 2012). Although there are no health complications seen due to the hypopigmentation in vitiligo patients but, there are several reports suggesting psycho-social complications in patients with vitiligo (Firooz *et al.,* 2004; Silverberg and Silverberg 2014). Affected people suffer from social stigma and girls, in particular, are subjected to ostracization from the marital point of view (Parsad *et al.,* 2003). The selective destruction of melanocytes from the epidermis results in the development of milky white patches. These patches, which are initially small, progressively increase in size and tend to form larger patches. Such patches may appear in any part of the body although the face, the back of the hands, wrists, axillae, umbilicus and genitalia are the regions that are most often affected (Kovacs, 1998).

It has been established that oxidative stress, the initial triggering factor, and autoimmunity are involved in the progressive melanocyte destruction in vitiligo (Laddha *et al.,* 2014). Compromised intrinsic antioxidant defence mechanisms in pathological conditions in addition to accumulation of ROS due to melanogenesis might make the epidermal melanocytes more vulnerable to oxidative stress (Denat *et al.,* 2014). Further, autoimmunity is strongly involved in the progression of disease, as around 30% of vitiligo cases are affected with at least one of the concomitant autoimmune disorders (Alkhateeb *et al.,* 2003). Anti-melanocyte antibodies, altered $CD4^+/CD8^+$ T cell ratio, imbalance of pro- and anti-inflammatory cytokines, etc. have been reported in vitiligo (Dwivedi *et al.,* 2015; Singh *et al.,* 2018). However, the mechanism by which oxidative stress activates autoimmunity remains obscure.

1.1.1 Vitiligo classification

Due to complexity in distribution of patches and differences in the etiopathogenesis of various types of vitiligo, a common well-defined nomenclature and disease classification was warranted. In 2007, the Vitiligo European Task Force (VETF) recommended definitions for the vitiligo (Taïeb *et al.,* 2007), however, a broader international consensus was required. Therefore, an approach for achieving broad international consensus was developed at The Vitiligo Global Issues Consensus Conference (VGICC) with the goal of guiding both clinical research and optimal patient management (Ezzedine *et al.,* 2012).

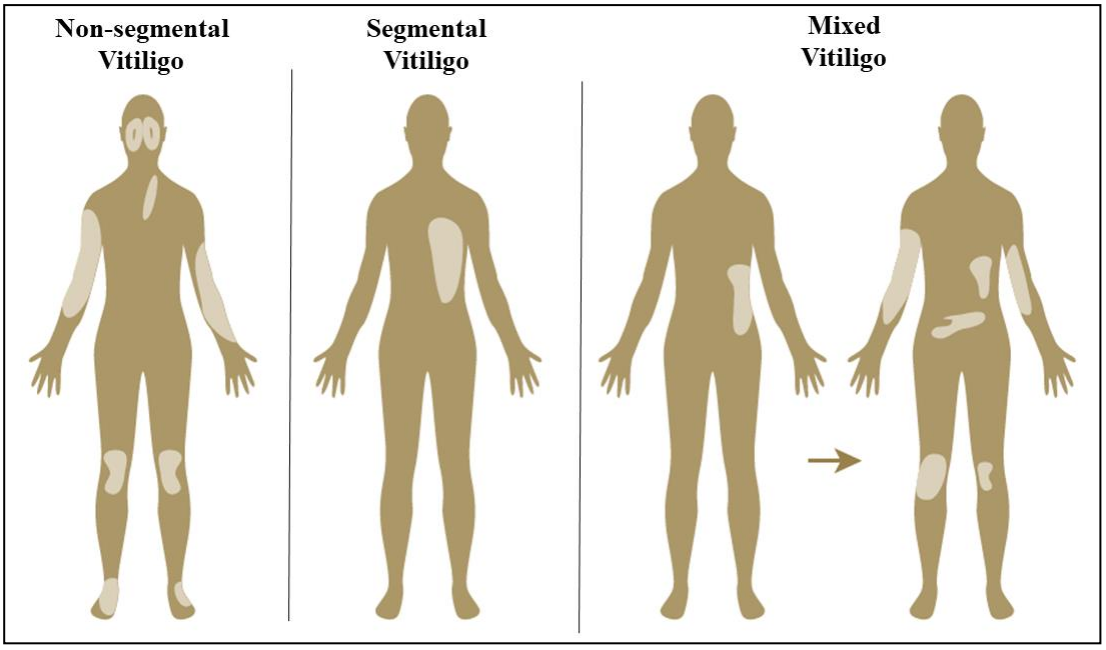

Figure 1.1: Vitiligo classification (Picardo *et al.,* 2015).

As per the revised classification, vitiligo has been classified into three main types: non-segmental, segmental and mixed vitiligo (Figure 1.1).

a) Non-segmental vitiligo (NSV): NSV can be characterized by depigmented macules that vary in size from a few to several centimetres in diameter, often involving both sides of the body with tendency toward symmetrical distribution. It includes further sub types such as acro-facial, generalized, mucosal (multifocal) and universal vitiligo. The onset of NSV is generally in later age and it is often associated with autoimmunity. Unstable results are commonly observed following autologous grafting in patients with this type of vitiligo (Taïeb and Picardo, 2019).

b) Segmental vitiligo (SV): Segmental vitiligo refers to a clinically unambiguous segmental distribution of depigmented lesions, typically associated with early onset and with leukotrichia. Its further sub types are focal, localized, mucosal, uni-segmental, bi- segmental or multi segmental vitiligo. It is rarely associated with autoimmune disorders and responds well to autologous grafting (Taïeb and Picardo, 2019).

c) Mixed vitiligo: The mixed type has an onset that is the same as segmental vitiligo but it then evolves into non-segmental vitiligo.

Further, based on the progression of the disease, vitiligo can be classified as active or stable vitiligo as proposed by Falabella *et al.,* (1995) and the Vitiligo Global Issues Consensus Conference (2012). The stable vitiligo is defined as the disease condition with lack of progression of old lesions within the past 2 years and no formation of new lesions within the same period. Active vitiligo (AV) is defined as the condition where there is appearance of new lesions and spreading of existing lesions during past two years duration (Falabella *et al.,* 1995; Ezzedine *et al.,* 2012).

1.2 Skin

Human skin is the body's largest organ, making up about 15% of adult body weight. Human skin is component of the integumentary system. Since skin is the body's most sensitive organ, it plays many essential roles including protection from external, physical, chemical, and biological attacks, as well as preventing surplus body water loss and also partly in thermoregulation. The mucous membranes surrounding the surface of the body are constant (Kanitakis, 2002), and the framework of the skin above the mucous membranes consisting of a complex network acts as an efficient barrier for preventing invasion of pathogens into the body. Thus, skin is one of the most important organs that gives the body innate immunity (Proksch *et al.,* 2008).

1.2.1 Epidermis

The epidermis is the topmost layer of skin. The epidermis is a ceaseless renewing layer that gives rise to derivative structures, such as pilosebaceous apparatus, nails, and sweat glands. Renewal of the outer epidermis is through proliferation cycles, which take place in the basal cells of the epidermis. It is a highly structurally defined and dynamically profound tissue, where cells are involved in unsynchronized interactions. The epidermis harbours a plethora of cell populations, such as melanocytes, Langerhans cells, and Merkel cells, but the

keratinocytes comprise most of the cells in the layer. According to keratinocyte morphology and position, the epidermis is divided into four layers (James *et al.*, 2006).

i. The basal cell layer (stratum germinativum)
ii. The squamous cell layer (stratum spinosum)
iii. The granular cell layer (stratum granulosum)
iv. The cornified or horny cell layer (stratum corneum)

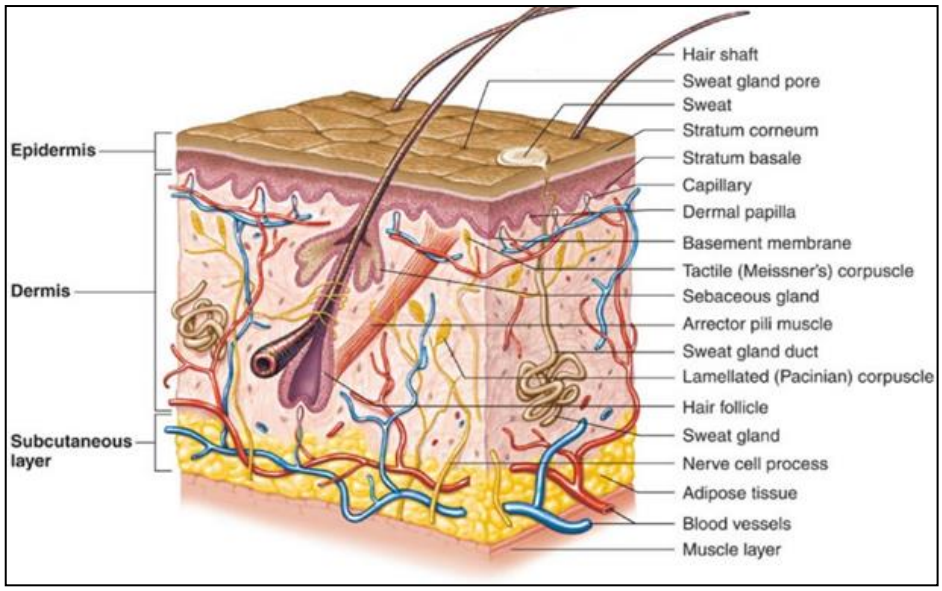

Figure 1.2: Cross section of human skin (Rees, 2003).

Cells in the Epidermis

Keratinocytes: These are the most abundant cells present in the epidermis, accounting for at least 80% of cells in the epidermis. Keratinocytes are ectodermal in their origin. As the cells migrate from the basal layer to the skin surface, they undergo differentiation, which leads to keratinization. The process of keratinization has two phases: synthetic and degradative phases (Chu, 2008). In the synthetic phase, the cell accumulates cytoplasmic supply of keratin, a fibrous intermediate filament arranged in an alpha-helical coil pattern serving as part of the cell's cytoskeleton. Keratin filament bundles cluster on and terminate at the plasma membrane developing the intercellular attachment plates known as desmosomes. In the event of the degradative phase of keratinization, cellular organelles are lost and the contents of the cell condense into a mixture of filaments forming a cornified cell envelope. Ultimately, the cell is known as horny cell or corneocyte. The process of corneocyte formation is known as terminal differentiation (James *et al.*, 2006).

Dendritic cells: Skin has a distinctive dendritic cell network mainly composed of embryo precursor-derived epidermal Langerhans cells and bone marrow-derived dermal conventional dendritic cells (cDCs), classified further as type 1 (cDC1) and type 2 (cDC2) subsets. Dendritic cells (DCs) are heterogeneous population of innate immune cells, which mediate immune responses by behaving as Antigen Presenting Cells (APCs) that present antigens to cognate T cells and stimulate other types of immune cells (Kim *et al.,* 2017).

Merkel cells: Merkel cells are examples of type I mechanoreceptors that are slow-adapting, oval shape cells situated at sites of high tactile sensitivity that are attached to basal keratinocytes with the assistance of the desmosomal junctions. These are found in the digits, lips, oral cavity regions, and outer root sheath of the hair follicle. These are assembled into specialized structures known as tactile discs or touch domes (Moll *et al.,* 1994). Even a relatively small deformation in the adjacent keratinocyte acts as a stimulus for Merkel cells to release a chemical signal stimulating an action potential in the adjoining afferent neuron relaying the signals to the brain. In areas with high tactile resolution and sensitivity, it is observed that there is a high concentration of Merkel cells, for e.g. the fingertips.

Langerhans cells: Langerhans cells are involved in a variety of T-cell responses and are derivatives from the bone marrow. In early embryonic development, these cells travel to supra-basal position in the epidermis where they continue to circulate and proliferate throughout life. Though these cells are dendritic, they are not involved in formation of cellular junctions with neighbouring cells. Langerhans cells make up 2%-8% of the total epidermal cell population and maintain nearly constant number and distribution in a particular area of the body. Langerhans cells are spread majorly in the squamous and granular layers of the epidermis. A few cells are found in the basal layer too. In addition to epidermis they are found in the oral cavity, esophagus and vagina, as well as in lymphoid organs and in normal dermis (Chu, 2008). Langerhans cells recognize and process soluble antigens present in epidermal tissue. When a membrane-bound antigen is endocytosed, cell granules are formed. The contents of these granules are delivered to phagolysosomes in the cytoplasm, which contain hydrolytic enzymes like those found in macrophages. In the first stage of life, the Langerhans cells are weak stimulators of un-primed T cells but can ingest and process antigens. Later, once the cell has become an effective activator of naive T cells, activation upon contact with the antigen does not trigger phagocytosis but rather stimulates cell migration (Udey *et al.,* 1997).

Melanocytes: Melanocytes are dendritic in nature. These are pigment synthesizing cells derived from the neural crest and are confined predominantly to the basal layer (Chu, 2008). Melanocytes encounter keratinocytes through branching in more superficial layers, however they do not form cellular junctions. Melanocytes are responsible for the synthesis of melanin and its transfer to keratinocytes. Melanin is synthesised in a rounded, membrane-bound organelle known as the melanosome through a series of receptor-mediated, hormone-stimulated, enzyme-catalysed reactions (Haake & Hollbrook, 1999). Melanosomes move to the end of the melanocyte processes underlying closest to the skin surface and are transferred to keratinocytes (Figure 1.3). In the regions of white skin melanosomes are grouped as membrane-bound melanosome complexes containing two or three melanosomes, whereas in dark skin areas, they tend to remain non-aggregated. The degree of melanisation is correlated with the size of the melanosomes and their associated release in keratinocytes (Flaxman *et al.*, 1973).

1.2.2 Dermis

The dermis is an integrated system of fibrous, filamentous and amorphous connective tissue that accommodates stimulus-induced entry by nerve and vascular networks, epidermally derived appendages, fibroblasts, macrophages and mast cells. The dermis involves the bulk of the skin and provides its pliability, elasticity and tensile strength. It protects the body from mechanical injury, binds water, aids in thermal regulation and includes receptors of sensory stimuli. The dermis interacts with the epidermis in maintaining the properties of skin. The two regions contribute during development in the morphogenesis of the dermal-epidermal junction and epidermal appendages and interact in repairing and remodelling the skin as wounds are healed. The matrix components like collagen and elastic connective tissue vary in a depth-dependent manner and undergo turnover and remodelling in normal skin during pathological conditions and in response to external stimuli (Chu, 2008). Collagen is the principal component of the dermis and a major stress-resistant material in the skin. Elastic fibres, on the other hand, are important in maintaining elasticity but do very little to resist deformation and tearing of the skin. Collagen represents 70% of the skin's dry weight and is also the most abundant protein in body (James *et al.,* 2006). Fibroblasts secrete procollagen, a specific helical polypeptide chain, which is secreted in extracellular space and organize into collagen fibrils. Collagen is highly enriched with glycine, hydroxyproline and hydroxylserine. The type I collagen is a major constituent of the dermis. Type IV collagen is found mainly in the basement membrane zone and the major structural component of anchoring fibrils is

collagen type VII, produced primarily by keratinocytes (James *et al.,* 2006). The elastic fibre is both structurally and chemically different from collagen and consists two components: protein filaments and elastin. The fibroblast conjoins elastic fibre to the extracellular matrix of the dermis made of glycosaminoglycans (James *et al.,* 2006).

1.2.3 Hypodermis

Hypodermis is the deepest layer of the skin. It consists of an extensive network of blood vessels, neurons and largely acts as a store house of adipose (Subcutaneous fat) tissue. The fat cells begin to develop in the subcutaneous tissue towards the end of the fifth month. Depending on the site where skin is present, the thickness of the panniculus region varies. Subcutaneous fat is considered an endocrine organ as it provides the body with buoyancy and functions as a storehouse of energy. The panniculus converts androstenedione into estrone by aromatase. Lipocytes produce leptin, a hormone that regulates body weight under regulation of the hypothalamus (James *et al.,* 2006).

1.3 The epidermal melanin unit

The epidermal melanin unit consists of one melanocyte contacting ~36 surrounding keratinocytes (Fitzpatrick *et al.,* 2011). The ratio of melanocytes to keratinocytes is 1:5 in the epidermal basal layer (Fitzpatrick, 1979). This balance is maintained through the human life, but the exact mechanism is unknown (Haass and Herlyn, 2005). For skin colour determination, successful contact between the dendritic processes of melanocytes and keratinocytes is vital for efficient transport of melanin to keratinocytes. Moreover, this contact is essential for photoprotection. Melanocytes, keratinocytes and dermal fibroblasts communicate with each other by secreted factors and by cell-cell contacts (Yamaguchi *et al.,* 2007). Keratinocytes monitor melanocyte growth and activity through paracrine growth factors and cell adhesion molecules (Haass and Herlyn, 2005; Lee, 2012). These cells behave as local source of the various hormones that control melanocyte proliferation, synthesis and dendrite formation (Sulaimon and Kitchell, 2003). Epidermal complex network involves crosstalk of several signalling pathways between keratinocytes and melanocytes that implicate in the maintenance of skin homeostasis.

Melanosome: Melanosome is an organelle found in melanocytes and is the site for synthesis, storage and transport of melanin. These pigment granules provide tissues with colour and photoprotection. Melanosome biogenesis has four stages (I–IV), which are determined by their quality, quantity, structure and arrangement of the melanin produced (Figure 1.3). They

are often divided as "early" (Stage I and II) and "late" melanosomes (Stage II and IV) according to lack or presence of pigment (Watabe *et al.,* 2008).

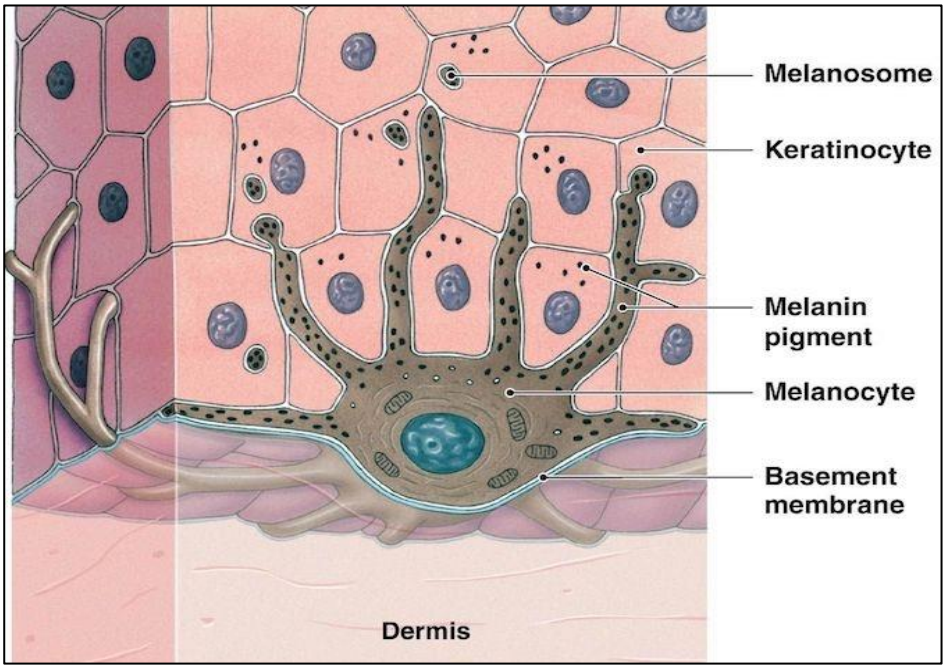

Figure 1.3: The epidermal melanin unit (Pozzo and Martini, 2015).

Melanosome Biogenesis:

Stage 1: The early melanosomes are found to have the proteins derived from the endoplasmatic reticulum, coated vesicles, lysosomes and endosomes. These structures are spherical vacuoles with no internal structural components.

Stage 2: The stage II shows visible fibrillar matrix formed by glycoproteins (Pmel17, MART-1), melanosomes elongate and tyrosinase is present.

Stage 3: In stage III, melanosomes begin melanin production, polymerize and settle on the internal fibrils. During this stage, melanosomes are of elliptical or ellipsoidal shapes.

Stage 4: In the last stage, melanosome is filled completely with melanin. Each type of melanin is synthesized in a separated melanosome. The eumelanosomes are large, elliptical, and contain a fibrillary matrix required for eumelanin production. The pheomelanosomes are smaller, spherical and their matrix has loose structure (Costin and Hearing, 2007).

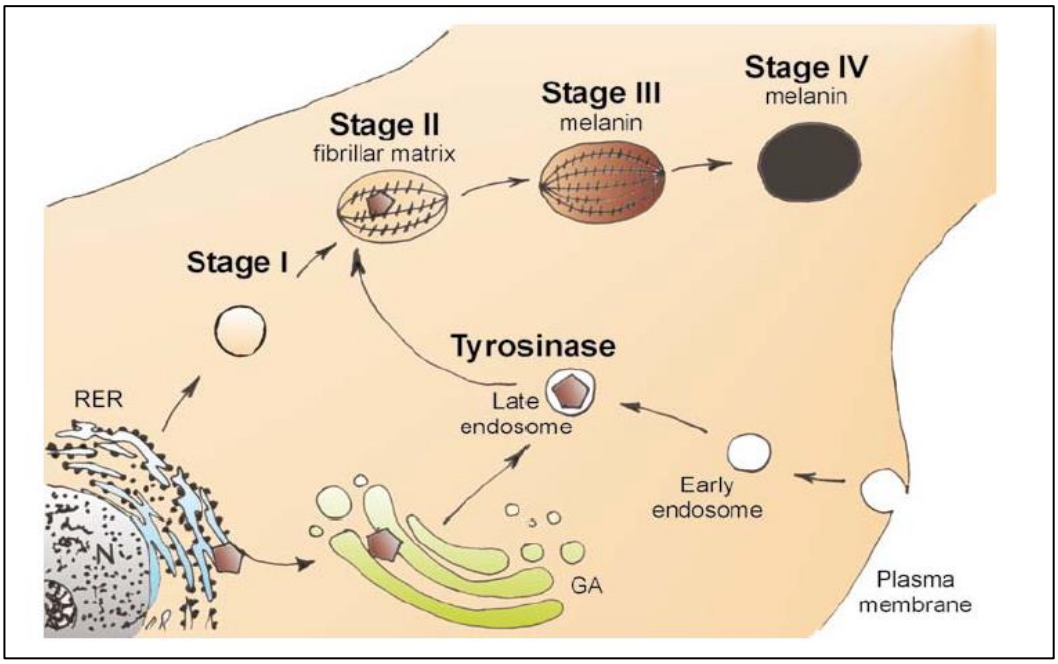

Figure 1.4: The melanosome formation and maturation during the melanin production by a melanocyte (Cichorek *et. al*, 2013).

1.4 Melanogenesis

Melanin is produced in membrane-bound granules called melanosomes, which are then transferred from melanocyte to the associated keratinocytes through its dendrites by phagocytosis. Melanin pigments are accumulated above the nucleus of keratinocyte and are removed with the shedding of epidermal cells. Melanin forms a shield over the skin protecting it against ultraviolet radiation damage that can cause skin cancer (Plonka *et al.*, 2009). The skin pigmentation is determined by melanin present in the keratinocytes. The melanocytes of dark brown- and black-skinned individuals produce more melanin of a darker colour than do the melanocytes of fair-skinned individuals. Skin colour is mainly determined by the mix of carotenoids, oxy-/deoxy-haemoglobin and most importantly, different types of melanin and also the way that melanin is packaged and distributed in melanosomes (Stamatas *et al.*, 2004; Jablonski, 2004).

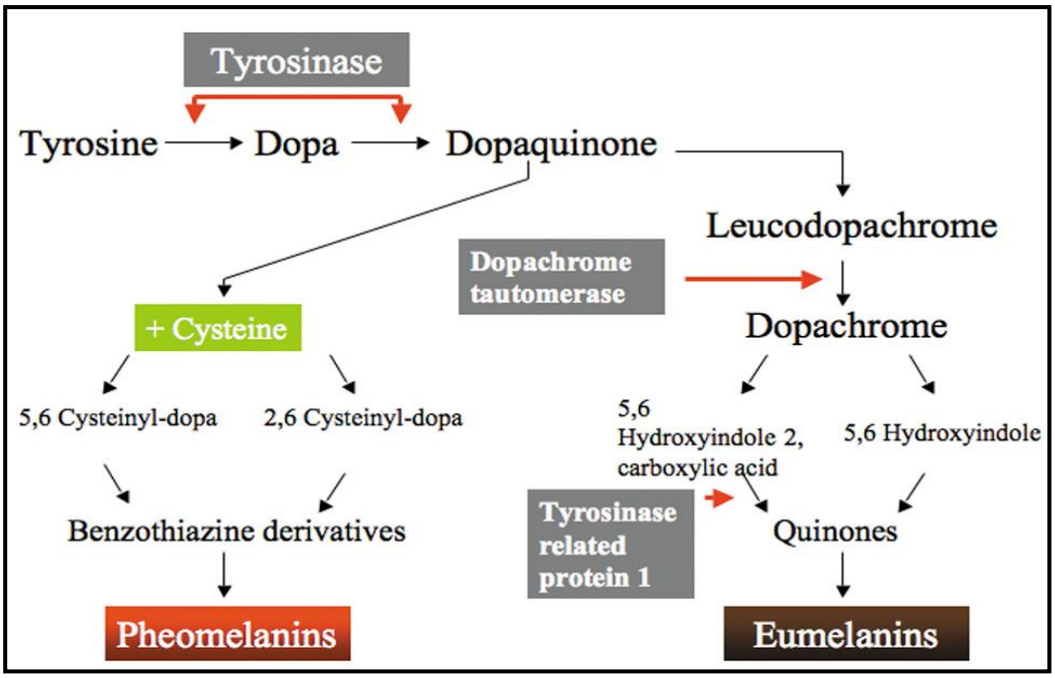

Figure 1.5: Melanin synthesis.

There are two types of melanin pigments viz., eumelanin (black-brown) and pheomelanin (red-yellow). Both melanins are derived from a common tyrosinase-dependent pathway with the same precursor, tyrosine. The obligatory step is hydroxylation of tyrosine to dopaquinone, from which L-DOPA can also be derived. From dopaquinone, the eumelanin and pheomelanin pathways diverge. Two enzymes crucial to eumelanogenesis are the tyrosine related proteins TRP1 (GP75) and TRP2 (dopachrome tautomerase, DCT). Pheomelanin is derived from conjugation of dopaquinone with thiol-containing cysteine or glutathione. As a result, pheomelanin is more photolabile and can produce, among its by-products, hydrogen peroxide, superoxide, hydroxyl radicals and all the trigers of oxidative stress, which can cause DNA damage. Melanosomes have association with microtubules and filaments via motor proteins, such as kinesin, dynein, myosinV that are important for melanosome movement along the dendrites and for subsequent transfer to keratinocytes (Slominski *et al.*, 2004).

1.5 Melanocytes loss in vitiligo

Vitiligo is characterised by depigmented milky white patches on the skin due to destruction of melanocytes from the epidermis. Melanocytes in hair follicles appear to be more robust, so that normal hair colour often persists in the white patch. Pigment-forming melanocytes are completely absent in lesional areas (Gilhar *et al.*, 1995; Le Poole and Boissy, 1997). However, there are evidences to suggest that even in long lasting disease, melanocytes still exist but have lost their integrity and functionality (Tobin *et al.*, 2000). Melanocyte death may

occur due to various intrinsic and/or extrinsic factors. Despite extensive research on vitiligo pathogenesis, the precise *modus operandi* for vitiligo pathogenesis has remained obscure. However, a complex interaction of genetic, environmental, biochemical and immunological events is likely to generate a permissive milieu (Laddha *et al.*, 2013b; Shoab Mansuri *et al.*, 2014; Singh *et al.*, 2018) for melanocyte loss. The crosstalk of various genes, that manipulate the autoimmune response, genetically abnormal melanocytes and one or more physiological or environmental factors that induce oxidative stress and activate the genetic program for melanocyte destruction, play an important role in the onset of the disease.

1.6 Genetic basis of vitiligo

The importance of genetic factors for vitiligo susceptibility is evident by reports of significant familial association from several studies (Zhang *et al.*, 2005; Shajil *et al.*, 2006; Spritz and Andersen, 2017). About 20% of vitiligo patients have at least one first degree relative affected and the relative risk of vitiligo for first degree relatives of vitiligo patients is increased by at least 7-10 folds (Bhatia, 1992). Vitiligo does not follow the simple Mendelian inheritance pattern of an autosomal dominant or autosomal recessive or X- linked inheritance (Casp *et al.*, 2002). Therefore, it has been proposed that vitiligo is a polygenic disease influenced by a set of recessive alleles occurring at several unlinked autosomal loci that collectively confer the vitiligo phenotype (Nath *et al*., 1994). Several studies have established the role of genetics in vitiligo susceptibility, however, the genetic risk is not absolute (Picardo *et al.*, 2015; Roberts *et al.*, 2019). Interestingly, monozygotic twins have only 23% concordance of the onset of disease (Spritz, 2013). Additional environmental factors are likely to influence vitiligo pathogenesis and affect the penetrance of the genetic predisposition to vitiligo. Genetic studies support both oxidative stress as well as immune mechanisms as the underlying factors for vitiligo pathogenesis. Our previous studies have identified several polymorphisms in genes involved in oxidative stress and immunity as susceptibility loci for vitiligo in Gujarat population.

Table 1.1: Genetic susceptibility loci identified in Gujarat population.

Sr. No	GENE	REFERENCE
1.	*Cytotoxic T-lymphocyte-associated protein 4 (CTLA4)*	Dwiwedi *et al*., 2011
2.	*Interleukin 4 (IL4)*	Imran *et al.,* 2012
3.	*Tumour Necrosis Factor alpha (TNFA)*	Laddha *et al*., 2012
4.	*Tumour Necrosis Factor beta (TNFB)*	Laddha *et al*., 2013
5.	*Melanocyte proliferating gene 1 (MYG1)*	Dwiwedi *et al*., 2013
6.	*NACHT, LRR, FIIND, CARD domain and PYD domains-containing protein 1 (NLRP1)*	Dwiwedi *et al*., 2013
7.	*Interferon- gama (INFG)*	Dwiwedi *et al*., 2013
8.	*Superoxide dismutase (SOD) 2, 3*	Laddha *et al*., 2013
9.	*Interleukin 1 beta (IL1B)*	Laddha *et al.,* 2014
10.	*Neuropeptide Y (NPY)*	Laddha *et al.,* 2014
11.	*Glutathione peroxidase (GPX)*	Mansuri *et al*., 2016
12.	*Catalase (CAT)*	Mansuri *et al*., 2017
13.	*Proteasome subunit beta type-8 (PSMB8)*	Jadeja *et al*., 2017
14.	*Methylenetetrahydrofolate reductase (MTHFR)*	Jadeja *et al*., 2018
15.	*Interleukin 1 Receptor Antagonist (IL1RN)*	Singh *et al.,* 2018
16.	*Glucose-6-phosphate dehydrogenase (G6PD)*	Mansuri *et al.,* 2019

1.7 Oxidative stress hypothesis

In general, oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage (Hensley *et al.,* 2000). ROS produced as byproducts of melanogenesis in melanocytes are controlled in the epidermis by several antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (Maresca *et al.,* 1997; Hensley *et al.,* 2000). The antioxidant mechanism repairs the damage caused by clearing the reactive intermediates formed due to oxidative stress (Hensley *et al.,* 2000; Nordberg and Arnér, 2001). The oxidative stress hypothesis suggests an imbalance in the level of pro- and anti-oxidants leading to accumulation of ROS. High levels

of SOD have been reported in blood of vitiliginous and non- vitiliginous skin of vitiligo patients as compared to controls (Koca *et al.,* 2004; Sravani *et al.,* 2009; Laddha *et al.,* 2013a). Several reports including our previous studies have shown significantly elevated SOD activity in the blood of vitiligo patients (Laddha *et al.,* 2013; Shajil *et al.,* 2006; Arican *et al.,* 2008). In addition, we have reported increased transcript levels of SOD2 and SOD3 in vitiligo patients (Laddha *et al.,* 2013). Glutathione peroxidase (GPX) converts H_2O_2 and other peroxides into H_2O. It protects the membranes and essential proteins from potential damaging effect of ROS and LPO (Halliwell, 1994). Hazneci *et al.,* (2005) have reported a significantly decreased levels of GPX in the epidermis of both lesional and nonlesional skin of active vitiligo patients. Earlier studies revealed decreased GPX activity in plasma and skin biopsies of vitiligo patients (Agrawal *et al.,* 2004; Beazley *et al.,* 1999). In addition, we found decreased catalase, G6PD and GPX activities in vitiligo patients (Agrawal *et al.,* 2004; Mansuri *et al.,* 2016, 2017, 2019). Various studies including ours have shown the increased LPO levels in vitiligo patients (Laddha *et al.,* 2014, Agrawal *et al.,* 2004). These findings suggest the role of oxidative stress in the pathogenesis of vitiligo and indicate that oxidative stress is not a very precise phenomenon but of a more generalized nature. This may be one of the reasons for developing new lesions in vitiligo patients (Sravani *et al.,* 2009). Oxidative stress can also lead to the activation of stress signalling pathways and transcription factors involved in inflammatory response and cell death mechanism (Fulda *et al.,* 2010).

1.8 Autoimmune hypothesis:

Autoimmunity plays a major role in the development of disease, as 30% of vitiligo cases are associated with at least one of the concomitant autoimmune disorders (Alkhateeb *et al.,* 2003). These associations indicate a common genetic etiological link between vitiligo and other autoimmune diseases (Passeron and Ortonne, 2005). Antibodies to melanocyte antigens are present in circulation of most of the vitiliginous patients (Naughton *et al.,* 1986; Cui *et al.,* 1992). The serum levels of antibodies to melanocyte antigens correlate with the activity and extent of disease (Ongenae *et al.,* 2003). Our studies also showed that ~75% of Gujarat vitiligo patients have anti-melanocyte antibodies in their circulation (Laddha *et al.,* 2014). Many proteins like tyrosinase (TYR), TRP1 and TRP2, OCA2, HERC2 and MC1R have been reported as melanocyte autoantigens in Caucasian population (Jin *et al.,* 2012). Histopathological investigations of the perilesional skin of vitiligo suggested the involvement of lymphocytes in the depigmentation process. Immuno-histochemical studies have confirmed the presence of infiltrating T cells (Le Poole *et al.,* 1996). It is detected that T cell

infiltrates with a predominant presence of $CD8^+$ T cells in generalized vitiligo (Abdel-Naser *et al.,* 1994; Badri *et al.,* 1993; Gross *et al.,* 1987; Wijngaard *et al.,* 2000). Several reports including ours have suggested a decreased $CD4^+/CD8^+$ ratio in vitiligo patients, indicating the prevalence of $CD8^+$ cells in patients (Grimes *et al.,* 1986; Halder *et al.,* 1986; Dwivedi *et al.,* 2013). Moreover, the increased pro- and decreased anti-inflammatory cytokines in blood as well as skin of vitiligo patients support the evidence of immune dysregulation in vitiligo pathogenesis (Figure 1.6) (Singh *et al.,* 2018).

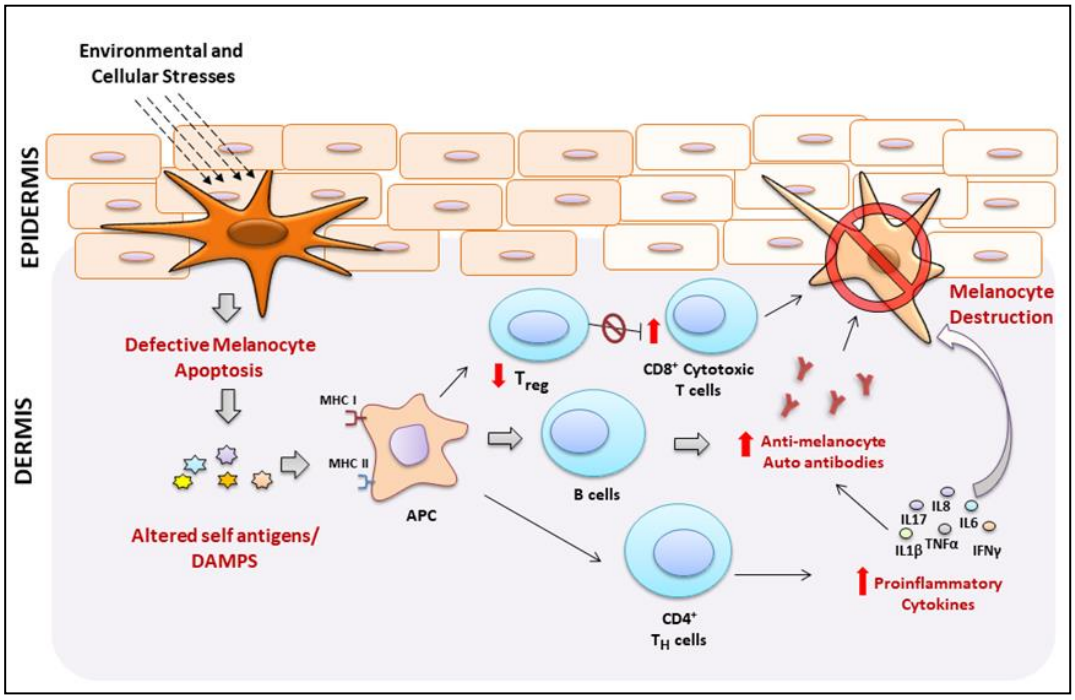

Figure 1.6: An overview of immune mediated destruction of melanocytes in vitiligo (Singh *et al.,* 2018).

1.9 Interplay among different pathomechanisms in vitiligo

Despite major scientific efforts, the exact pathophysiology of melanocyte destruction in vitiligo remains elusive. A convergence theory was put forward to incorporate the existing theories of vitiligo onset into a single overview of vitiligo pathogenesis (Poole *et al.,* 1993). It suggest that the melanocyte viability is affected by multiple factors such as the predisposing genetic loci, chemical/radiation exposure, or hyper reactive T cells, but rather results from a combination of etiologic factors that impact melanocyte viability (Poole *et al.,* 1993; Kundu *et al.,* 2019). Various pro-oxidants are generated during melanin synthesis and compromised intrinsic antioxidant defence mechanisms make epidermal melanocytes vulnerable to oxidative stress (Denat *et al.,* 2014). Oxidative stress triggers vitiligo onset, while

autoimmunity contributes to the disease progression (Laddha *et al.,* 2014). However, the exact mechanism that connects the triggering factors with the disease progression is still obscure. Based on previous literature we have speculated that the ER stress could be a potential link between oxidative stress and autoimmunity (Mansuri *et al.,* 2014) where oxidative stress might cause accumulation of misfolded proteins leading to ER stress. This activates intracellular signalling pathway known as unfolded protein response (UPR) to resolve the protein-folding defect. When there is chronic or severe ER stress, or compromised UPR, apoptotic signalling pathways are activated. UPR induces the production of various pro-inflammatory cytokines such as TNF-α, IL-6, IL-8, IL-1β, IL-23, etc. (Garg *et al*., 2012). Furthermore, UPR can contribute to the activation of autoimmune response by generation of altered antigens during degradation of misfolded proteins, besides the release of neo-antigens by apoptotic cells, and altered immune-tolerance in cells with an anomalous UPR (Lipson *et al.,* 2006; Todd *et al.,* 2008).

1.10 Endoplasmic Reticulum (ER) stress

The endoplasmic reticulum (ER) is an active intracellular organelle with different functions like protein folding and maturation within the eukaryotic cell essential for cellular homeostasis, proteostasis, cellular development and stress responsiveness (Ramirez *et al.,* 2020). Accurate protein folding is necessary for cell survival, and various mechanisms make sure that the correctly folded, modified and assembled proteins are transported to their site of function (Bravo *et al.,* 2013). The newly translated polypeptide contains an N-terminal sequence that is first recognized by signal recognition particle (SRP). SRP allows its entry to the ER through the translocon complex; after it enters inside the ER, the signal sequence is cleaved by signal peptidase and the polypeptide sequence undergoes post-translational modifications (Rapiejko and Gilmore, 1992). In the ER, glycosylating enzymes alter the glycan chain either trim or add N-linked oligosaccharides for recognition of nascent polypeptide and work as a quality control step (Wang and Kaufman, 2016; Hetz and Papa, 2018). If the polypeptide chain fails to adopt its native conformation, it may activate the ERAD (ER-associated degradation) pathway. In this situation, the nascent polypeptide undergoes degradation by 26 proteasome, or gains its natural conformation and transits further (Hampton, 2002). ER is a major site of synthesis and transport of a variety of biomolecules, it is also a major store house of intracellular Ca^{2+} (Schwarz *et al.,* 2015). The typical cytosolic concentration of Ca^{2+} is ~100 nM, while the Ca^{2+} concentration in the lumen of the ER is 100–800 μM and the extracellular Ca^{2+} concentration is ~2 mM (Samtleben *et*

al., 2013). High Ca^{2+} concentration is also necessary for the formation of disulfide bonds, which is essential for gaining tertiary and quaternary protein structure. The ER is abundantly supplied with Ca^{2+} dependent molecular chaperones like Hsp70-type chaperone, BiP (immunoglobulin binding protein) (Ma and Hendershot, 2004). Inside the endoplasmic reticulum, the protein folding occurs with the help of ER resident enzymes and chaperones; they help in post-translational modifications and boost the productivity of the protein folding of the nascent polypeptide. BiP helps the nascent polypeptide to achieve its native structure and also inhibits its aggregation and maintains them in a foldable state (Ma and Hendershot, 2004; Ni and Lee, 2007). The protein folding requirement varies from cell to cell; for secretory cells, such as plasma cells, insulin-producing β cells, melanocytes, and cancer cells, which have increased protein synthesis rates, the demand for the high protein folding is much higher than that for the normal cell (Bravo *et al.,* 2013). Sometimes ER can overrun the protein-folding mechanism because of the high demand for protein production in presence of various stressors like inflammation, microbial infection, radiation, hypoxia shock, heat, oxidation, nutrient deprivation, energy disturbance, calcium depletion and oxidative stress. (Kitakaze and Tsukamoto, 2010; Toko *et al.,* 2010). Also, certain situations like improper folding, insufficient glycosylation, inhibited transport and inhibited ERAD, may result in the accumulation of misfolded proteins (Schiano *et al.,* 2015). Multiple biological insults such as calcium and redox imbalance, viral infection, oxidative stress, and decrease in ATP pool lead to perturbation of ER homeostasis, which consequently initiates the accumulation of unfolded proteins in the ER lumen which is known as ER stress (Maly *et al.,* 2014).

1.10.1 ER stress induced Unfolded Protein Response (UPR) signalling:

In order to combat and alleviate ER stress, the cell activates Unfolded Protein Response (UPR). UPR reduces ER stress through global translation attenuation, induction of chaperones, degradation of misfolded proteins by ERAD and induction of apoptosis following unresolved or sustained ER stress (Schuck *et al.,* 2009) (Figure 1.7). The accumulation of misfolded proteins increases the production of BiP/GRP78 (78-kDa glucose-regulated protein) (Kozutsumi *et al.,* 1988). GRP78 forms the dynamic stability between nascent polypeptide (unfolded protein) and intra-luminal domains of the three ER stress sensors namely inositol-requiring enzyme 1α (IRE1α), PKR like endoplasmic reticulum kinase (PERK)(Huang *et al.,* 2006), and activating transcription factor 6 (ATF6) (Wang *et al.,* 1998; Haze *et al.,* 1999; Huang *et al.,* 2006; Mu *et al.,* 2008). In non-stress condition, all three

sensors are primarily bound with GRP78, which help to maintain its inactive state (Chen *et al.*, 2002; Oakes and Papa 2015) (Figure 1.8).

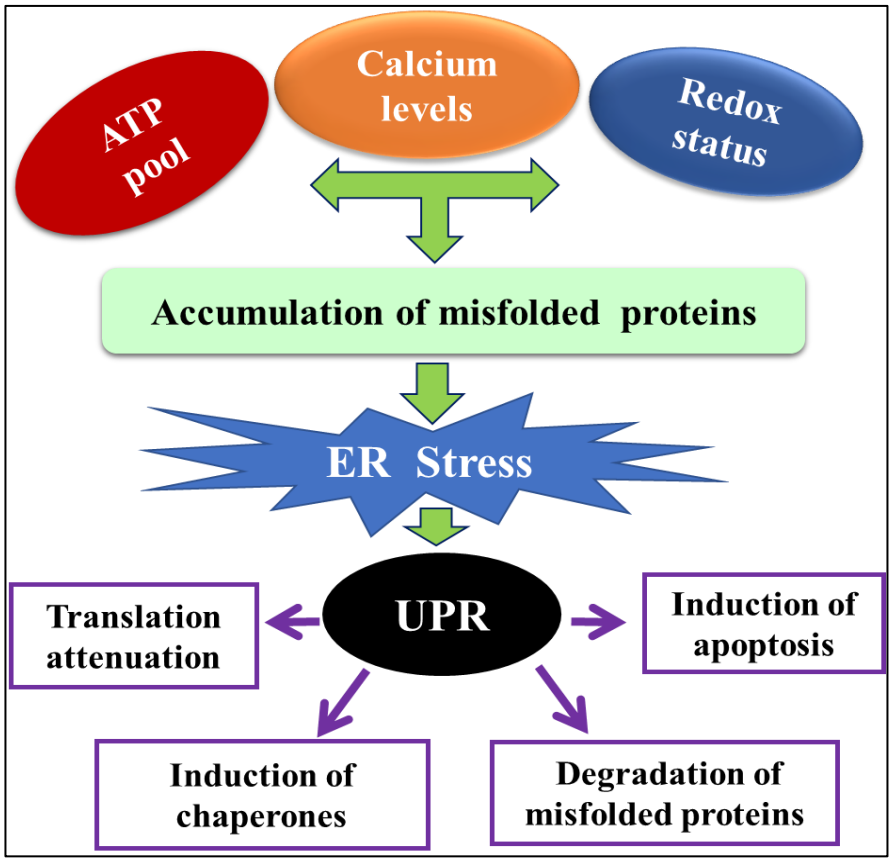

Figure 1.7: Functional aspects of ER stress induced UPR.

***Activating transcription factor 6 (ATF6) pathway*:** Activating transcription factor 6 is a type two transmembrane transcription factor, and it is also recognized by a cAMP-responsive element-binding protein/ATF basic leucine zipper domain (Haze *et al.*, 1999). After release from the GRP78, ATF6 does not undergo oligomerization and autophosphorylation but it translocates to the Golgi complex, where it is cleaved by the Golgi-localized site-1 and site-2 proteases (S1P and S2P) (Zhang and Wang 2012). Translocation of ATF6 to the Golgi is essential for its activation (Morishima *et al.*, 2011). Juxtamembrane cleavage of ATF6 further results in its release into the cytosol (Xu *et al.*, 2005). The cytosolic 50-kDa fragment/cytosolic basic leucine zipper (bZIP) domain of ATF6 translocates to the nucleus and binds with the ATF/cAMP response elements (CRE) and endoplasmic reticulum stress response element (ERSE-1) thus mediating the activation of UPR targeted genes such as a XBP1, CHOP and GRP78 (Basseri and Austin 2012; Thorp 2012; Sano and Reed 2013) (Figure 1.8). ATF6 is also a crucial activator of the expression of genes encoding molecular chaperones and elements of ERAD (Adachi *et al.*, 2008). ERAD (ER-associated degradation)

is a very complex system where misfolded/ unfolded proteins are linearized, retro-translocated to the cytosol, ubiquitinated, and transferred to the proteasome for degradation. It demonstrates an efficient strategy for the clearance of the accumulated unfolded and misfolded proteins in the ER lumen (Meusser *et al.*, 2005; Morito and Nagata, 2015). Overall, ATF6 downstream signalling leads to increased protein synthesis, increases the chaperone protein availability, enhances the ERAD, which reduces the density of unfolded proteins and subsequently leads to cell survival (Li *et al.*, 2000; Zhang and Kaufman, 2004). Following a prolonged ER stress, ATF6 can also induce CHOP expression and thus contributing to UPR-related cell death (Iurlaro and Muñoz-Pinedo, 2016; Bartoszewski *et al.*, 2019).

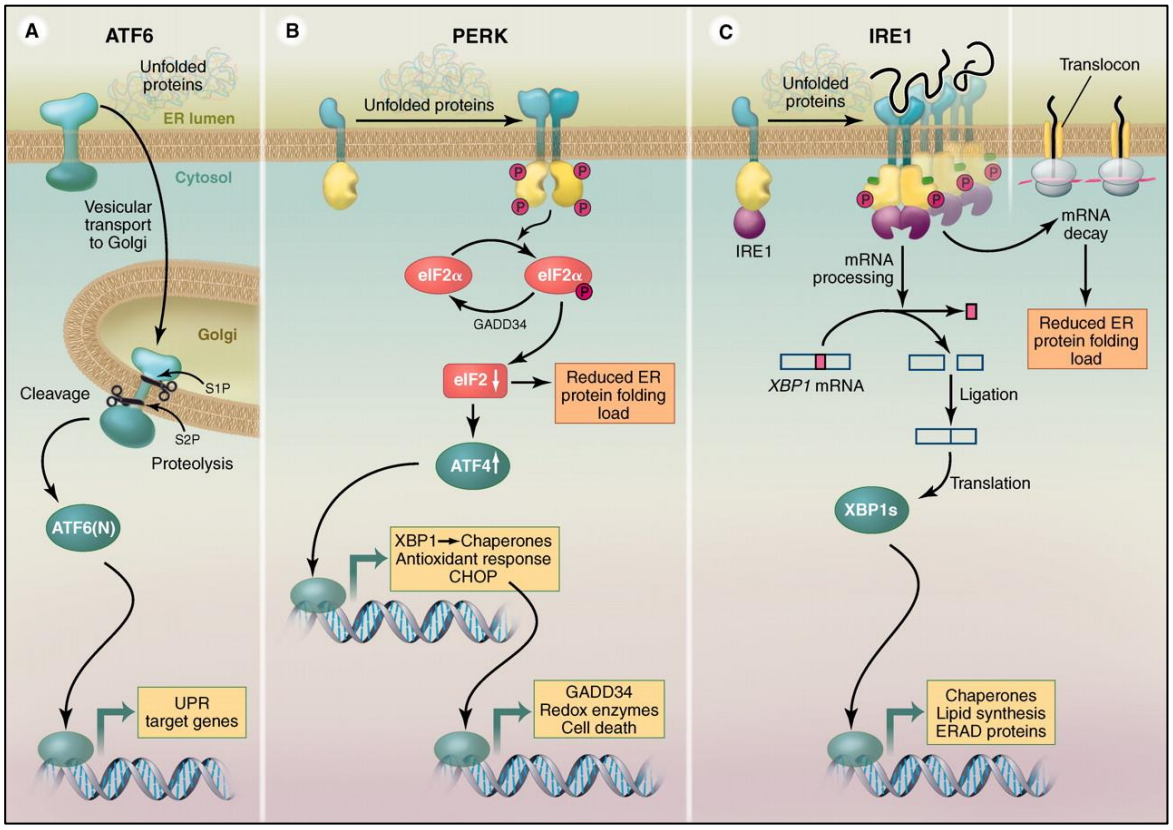

Figure 1.8: An overview of ATF6, PERK and IRE1 mediated UPR signalling (Walter and Ron 2011).

Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) pathway: Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) is also known as eukaryotic translation initiation factor 2-alpha kinase3 (EIF2AK3). Dissociation of GRP78, induces oligomerization and phosphorylation of PERK (Figure 1.8). Further, PERK in turn phosphorylates eukaryotic translation initiation factor 2α (eIF2α), preventing the formation of the ribosomal pre-

initiation complexes and lowering the cap-dependent protein translation which will result in a global protein translation attenuation (Walter and Ron, 2011). This allows the cell to efficiently fold the ER lumen proteins that are already present there (Sano and Reed, 2013). Due to the drastic fall in the amount of active eIF2α, the translation of the transcription factor ATF4 (activating transcription factor 4) is upregulated (Shi *et al.,* 1998). ATF4 translocates to the nucleus and binds with the unfolded protein response element (UPRE), which results in transcriptional changes in UPR target genes, including CHOP (C/EBP homologous protein; also known as GADD153) and DNA damage-inducible protein (GADD34) (Lei *et al.,* 2017; Lumley *et al.,* 2017). Activation of CHOP, in early ER stress results in the induction of pro-survival pathways and activation of a number of ameliorating genes including *XBP1* and chaperones; that subsequently leads to an antioxidant response, increases ERAD, amino-acid biosynthesis, and promotes the protein folding and maintains redox homeostasis to sustain cell survival (Rutkowski and Kaufman 2003). Notably, GADD34 mediated dephosphorylation of eIF2 allows the restoration of protein synthesis upon ER stress recovery (Novoa *et al.,* 2001). If the ER stress persists for an extended period, then it leads to cell cycle arrest and further to cell death via initiating the pro-apoptotic pathway by increasing the expression of CHOP (Oyadomari and Mori 2004).

Inositol-requiring enzyme (IRE1) pathway: Inositol-requiring enzyme 1 (IRE1) is also known as endoplasmic reticulum to nucleus signalling 1 (ERN1). IRE1 initiates the highly conserved arm of UPR signalling. IRE1 is also a ER transmembrane protein with a protein kinase and ribonuclease domain that extends through the cytoplasmic region (Walter and Ron 2011). IRE1 oligomerizes and autophosphorylates upon dissociation from GRP78. This phosphorylation activates the endonuclease domain, which shows the endoribonuclease activity by splicing of an intron from the 26-nucleotide sequence of *XBP1* mRNA resulting in spliced XBP1 (XBP1s) with the enhanced internal ribosome entry site (IRES) (Yoshida *et al.,* 2001). Further, it leads to IRES dependent translation of sXBP1 mRNA and the activation of UPR target genes such as chaperones, *CHOP*, *XBP1,* endoplasmic reticulum oxidoreductin-1 (*ERO1*), ER degradation-enhancing α-mannosidase-like protein (*EDEM*) etc. consequently leading to increased protein folding, lipid synthesis, ERAD capacity (Toth *et al.,* 2007; Bartoszewski *et al.,* 2011). The RNase domain of IRE1 also controls the endoribonuclease activity, which causes mRNA decay to reduce the burden of misfolded proteins within the ER, a process called IRE1 dependent decay (RIDD) (Figure 1.8). Degradation of ribosomal-associated mRNA prevents the translation and further accumulation of unfolded/misfolded

proteins and directs toward cell survival (Orr *et al.,* 2006). Under prolonged ER stress, excessive IRE1 activation can degrade some anti-apoptotic pre-microRNAs through RIDD (Han *et al.,* 2009; Hollien *et al.,* 2009; Upton *et al.,* 2012). Also, during persistent ER stress, the IRE1 activates its kinase function by joining with TNF receptor-associated factor 2 (TRAF2) and apoptosis signal-regulating kinase1 (ASK1), which further results in phosphorylation of c-Jun N-terminal Kinase (JNK). This further activates the pro-apoptotic BIM and inhibit anti-apoptotic BCL-2, leading to the cell death (Ron and Hubbard, 2008; Si *et al.,* 2008; Chen and Brandizzi, 2013).

1.11 ER stress and oxidative stress

Crosstalk between ER stress and oxidative stress has been established in many physiological and pathological conditions (Malhotra and Kaufman, 2007). The major enzymatic ROS contributors during the ER stress are protein disulfide isomerase (PDI), endoplasmic reticulum oxidoreductin 1 (ERO-1), and NADPH oxidase complexes, additionally mitochondrial electron transport enzymes also produce ROS (Santos *et al.,* 2009). The formation of disulfide bonds in ER resident proteins are driven by PDI and ERO1. PDI accepts electrons from protein-folding substrates, thereby oxidizing the thiol group in protein cysteine residues resulting in the formation of disulfide bonds. ERO1 mediates the transfer of electrons from PDI to molecular oxygen, leading to the production of reactive oxygen species (ROS) (Tu *et al.,* 2004). Further, ER stress as well as oxidative stress both, through ROS generation, lead to excessive Ca^{2+} leakage from the ER lumen. The resulting increase in the cytosolic Ca^{2+} level elevates mitochondrial ROS production (Berridge *et al.,* 2003; Gorlach *et al.,* 2006). Increased ROS production within the mitochondria further escalates Ca^{2+} release from the ER (Jacobson and Duchen, 2002). Moreover, ROS can also feedback to sensitize the ER membrane Ca^{2+} release channels resulting in diminished antioxidation potential of the cell which makes the Ca^{2+} release and ROS production cycle threatening for cell survival (Berridge, 2002, Vineri *et al.,* 2000). CHOP is a major pro-apoptotic factor of the UPR, which induces oxidative stress in various manners. CHOP activates ERO1 expression and thereby increases ROS production during ER stress. Additionally, ERO1 may cause inositol-1,4,5-trisphosphate receptor (IP3R)-mediated ER Ca^{2+} release, which activates Ca^{2+} sensing kinase (CaMKII) in the cytosol, leading to the activation of pro-apoptotic signalling (Cao and Kaufman, 2012, Hetz, 2012, Tabas and Ron, 2011). CaMKII can also induce Nox2 causing oxidative stress, which in turn results in PERK dependent CHOP activation during ER stress (Li *et al.,* 2010). Interestingly, due to the deleterious effect of oxidative stress induced protein

misfolding, eukaryotic cells have developed antioxidant stress responses to restore the cellular redox homeostasis. The PERK arm of the UPR induces two transcription factors ATF4 and NRF2, that can trans-activate the antioxidative stress response genes (Santos *et al.*, 2010). In addition, small molecular antioxidants, for e.g., butylated hydroxyanisole (BHA), can attenuate the ER stress-induced apoptosis and facilitate proper protein folding and secretion, which further reveals the crucial role of oxidative stress and ER stress cross talk (Malhotra and Kaufman, 2011; Cao and Kaufman 2014).

1.12 ER stress and Immunity

Various studies have discovered that ER stress induced UPR may induce inflammatory response. ER stress signalling generally leads to inflammation in order to regulate the tissue damage and help in tissue repair. However, ER stress-induced inflammation may also aggravate leading to several pathological conditions like diabetes, rheumatoid arthritis, atherosclerosis and cancer (Garg *et al.,* 2012). All three main arms of the UPR (PERK, IRE1α, and ATF6) can orchestrate pro-inflammatory transcriptional regulation, through key transcription factors like NF-kB and AP-1 (Hotamisligil and Erbay, 2008; Zhang and Kaufman, 2008; Verfaillie *et al.,* 2010). NF-kB is one of the central mediators of pro-inflammatory pathways. Genes transcribed by NF-kB include those encoding crucial pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-6, IL-8, IL-1β, IL-23 and IL-17 (Li *et al*., 2005; Zhang *et a*l., 2008; Pahl *et al.,* 1999). Although all the three UPR arms can activate NF-kB, they operate via different mechanisms. The IRE1 pathway activates NF-kB through its interaction with TRAF2 that will help in activation of IKK. Activated IKK will further results in IkB degradation, making NF-kB free for nuclear translocation (Hu *et al.,* 2006; Kaneko *et al.,* 2003). Activation of PERK causes attenuation of protein translation, which results into decreased levels of IkB and a consequent increase in the NF-kB/IkB ratio. This change in the ratio consequently releases NF-kB for its transcriptional activity (Obeid *et al.,* 2007; Ghiringhelli *et al.,* 2009). ATF6 activation can also cause NF-kB activation though the mechanism is not yet clear, however, cleaved ATF6 fragment acts as a transcription factor and reported to be involved in regulation of acute-phase response (APR)-associated genes, (Zhang *et al.,* 2006; Yamazaki *et al.,* 2009). Interestingly, increasing evidences suggest that ER stress is also involved in regulation of cell apoptosis, cytokine production, cell differentiation, antibody production, mitochondrial function and Toll-like receptor (TLR) signalling of various immune cells (Reverendo *et al.,* 2019; Li *et al.,* 2020).

1.13 Role of ER stress in vitiligo pathogenesis

Dialated ER is a marker for ER stress, and dilated ER was observed in perilesional skin biopsies as well as in melanocytes cultured from vitiligo patients (Boissy *et al*., 1991, Le poole *et al*., 1997 and 2000). Vitiligo patients exhibit very high levels of H_2O_2 and peroxy nitrite in their lesional skin, with concomitantly reduced catalase levels (Maresca *et al*., 1997; Schallreuter *et al*., 1999; Schallreuter *et al*., 1991). It was reported that H_2O_2 induced ER dilation hinders the export of tyrosinase from the ER of melanocytes (Guan *et al*., 2015). Lu *et al*., (2016) have shown bilobalide, a plant extract having antioxidant properties, protects human melanocytes from oxidative damage by inhibiting H_2O_2 induced apoptosis and inhibiting ER stress. ER stress-induced UPR signalling is also associated with the production of various pro-inflammatory molecules such as TNF-α, IL-6, IL-8, IL-1β, IL-23, Monocyte Chemoattractant Protein (MCP)-1, etc. (Todd *et al.,* 2008; Garg *et al*., 2012). Toosi *et al*., (2012) have reported that vitiligo-inducing phenols activate UPR in melanocytes and upregulates the expression of IL-6 and IL-8. In addition, inhibitors of *XBP1* reduce IL-6 and IL-8 production induced by phenols (Toosi *et al.,* 2012). Interestingly, association of *XBP1* polymorphisms and increased expression of *XBP1* has been observed in lesional skin of vitiligo patients (Ren *et al.,* 2009), emphasizing its involvement in ER stress and autoimmune mediated melanocyte destruction. We have shown a significant increase in *CHOP* and *IL23* expression in the skin of vitiligo patients providing an evidence of ER stress induced inflammation in vitiligo (Mansuri *et al.,* 2016). Expression of pro-inflammatory cytokines such as IFN-γ (Watanabe *et al.,* 2003), IL-1β (Akerfeldt *et al.,* 2008, Gurzov, *et al.,* 2009), TNF-α, IL-6 (Zhang, *et al.,* 2006), IL-17 (So *et al.,* 2015) by immune cells can generate further ER stress. Xin *et al.,* (2005) have observed that TNF-α induces the UPR in a ROS-dependent fashion and leads to cell death (Xin *et al.,* 2005). In addition, Li *et al.,* (2016) have demonstrated that increased expression of CXCL16 by impaired keratinocytes in response to oxidative stress could play an important role in $CD8^+$ T-cell skin migration in vitiligo patients. Moreover, the CXCL16 expression in keratinocytes was induced due to oxidative stress via two UPR pathways i.e., PERK-eIF2a and IRE1a-XBP1 (Li *et al.,* 2016). Thus, several direct and indirect evidences suggest ER stress to be a missing link between oxidative stress and autoimmunity in vitiligo pathogenesis.

Table 1.2: Interplay of cytokines and oxidative stress in vitiligo.

Sr. No.	Cytokine	Regulated via ER stress	Regulated via Oxidative stress	Levels in vitiligo	References
1.	TNF-α	Yes	Yes	Increased	Xin *et al.,* 2005; Laddha *et al.,* 2012
2.	IFN-γ	Yes	Yes	Increased	Dwivedi *et al.,* 2013; Gurzov*et al.,* 2009
3.	IL-6	Yes	Yes	Increased	Toosi *et al.,* 2012 (Unpublished data)
4.	IL-8	Yes	Yes	Increased	Toosi *et al.,* 2012 Farag *et al.,* 2017
5.	IL-1β	Yes	-	Increased	Laddha *et al.,* 2014; Gurzov*et al.,* 2009
6.	IL-23	Yes	-	Increased	Garg *et al.,* 2012; Mansuri *et al.,* 2016
7.	IL-17	Yes	-	Increased	Kotobuki *et al.,* 2012; So *et al.,* 2015 (Unpublished data)
8	CXCL16	Yes	Yes	Increased	Li *et al.,* 2016

1.14 Translational relevance of ER stress in vitiligo

From the above, it is clear that ER stress could be at the interphase of oxidative stress and inflammatory/immuno regulatory response in the cell, making it an ideal therapeutic target. However, the core UPR signalling involved in melanocyte biology and vitiligo pathomechanisms are not much explored. A few studies demonstrate that therapeutic agents modulating ER stress can be promising for vitiligo treatment. Recently, Zhu *et al.,* (2019) have reported that Baicalin attenuated the progression and reduced the area of depigmentation in C57BL/6 mouse model of vitiligo. Furthermore, Baicalin stimulated the proliferation of melanocytes in depigmented skin and also leads to decrease in $CD8^+$ T cell infiltration and the expression of CXCL10 and CXCR3 in vitiligo mouse skin. Interestingly, significantly decreased cytokines (IL-6, TNF-α, IFN-γ, and IL-13) levels in sera of vitiligo mouse model are reported (Zhu *et al.,* 2019). Baicalin is an active ingredient of *S. baicalensis*, which is a traditional Chinese herbal medicine having several pharmacological properties, like,

antioxidant, anti-tumor and anti-inflammation (Gao *et al.*, 2001; Shen *et al.*, 2003). Though, Zhu and colleagues did not show, but other studies have demonstrated that the antioxidant and anti-inflammatory activities of baicalin are through modulation of ER stress response. Shen *et al.,* (2014) observed that baicalin protected cardiomyocytes from ER stress-induced apoptosis via downregulating CHOP expression. Cao *et al.*, have reported that baicalin decreased the H_2O_2 induced cytotoxicity and oxidative stress in chondrocytes and pre-treatment of baicalin downregulated the expression of ER stress marker genes GRP78 and CHOP in H_2O_2 treated chondrocytes (Cao *et al.,* 2018). Another compound bilobalide is one of the active components of *G. biloba* extract. Lu *et al.,* (2016) have reported that pre-treatment with bilobalide could protect human melanocytes (NHM) from oxidative damage by inhibiting H_2O_2 induced cytotoxicity. Bilobalide showed inhibition of eIF2α phosphorylation and downregulation of CHOP expression and it also induced the expression of anti-oxidant genes such as *CAT* and *GPX*. A recent study on PIG1 cells (immortalized primary normal human melanocytes) also demonstrated that *Ginkgo biloba* extract could protect the cells from H_2O_2 induced oxidative stress by Nrf2 activation (Zhang *et al.,* 2019). However, the exact mechanism of ER stress modulation by these herbal extracts is not clear. Apart from these, therapeutic strategies aiming to improve protein-folding capacity during ER stress might also be promising. Chemical chaperones such as Tauro-ursodeoxycholic acid (TUDCA) and 4-phenyl butyrate (PBA) are ER stress inhibitors and improve the protein folding in the ER. A success in alleviation of ER stress-induced hyperglycaemia, restoration of insulin sensitivity and fatty liver disease amelioration was observed upon TUDCA and 4-PBA treatment in obese mouse (Ozcan *et al.,* 2006). Cao *et al.,* (2013) have reported that TUDCA and 4-PBA decreased ER stress in the intestinal epithelium leading to reduced dextran sodium sulfate (DSS)-induced colitis severity. Moreover, it was found that 4-PBA lead to decrease in lipopolysaccharide (LPS)-induced lung inflammation through modulating ER stress, nuclear factor κB (NF-κB) and hypoxia-inducing factor 1α (HIF1α) signalling (Kim *et al.,* 2013). Nevertheless, further studies to understand the molecular mechanism of ER stress signalling in melanocytes, neighbouring keratinocytes and circulatory as well as infiltrated immune cells are warranted for the development of novel targeted and personalised ER stress modulating therapeutics for vitiligo.

In view of the above, an interplay of oxidative stress, ER stress and autoimmunity in genetically predisposed individuals is implicated in vitiligo pathogenesis. Though the role of oxidative stress in vitiligo pathogenesis is well addressed the underlying mechanism about

ER stress in the development of inflammatory and autoimmune response resulting in melanocyte destruction is unclear. Hence, it would be interesting to investigate the factors connecting ER stress and autoimmunity leading to melanocyte destruction in vitiligo.

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