REGULATION OF MELANOCYTES IN MELANOMA AND VITILIGO

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Abstract: Melanoma is the malignant tumor of melanocytes and melanocytes are the cells that produce the dark (brown-black) pigment, melanin, which is responsible for the color of skin. Vitilgo is another skin disease related to depigmentation. A model has been proposed for the plausible role of MITF as a regulator of melanoma and vitiligo depending on the input stimulus/stimuli and internal environment, which governs this transcription factor to either maintain homeostasis or result to abnormality.

Index Terms: Melanoma, MITF, vitiligo, transcription factor.

1. INTRODUCTION:

1.1 Melanoma: Melanoma is one of the most aggressive skin cancers with highest mortality rate. Melanoma is the malignant tumor of melanocytes and melanocytes are the cells that produce the dark (brown-black) pigment, melanin, which is responsible for the color of skin. Extrafollicular dermal melanocyte stem cells *(MSCs)* persist after birth in the superficial nerve sheath of peripheral nerves and give rise to migratory melanocyte precursors when replacements for epidermal melanocytes are needed on the basal epidermal layer of the skin. The detrimental effects of UV radiations on DNA and repair mechanism in melanocyte stem cell convert them to melanoma stem cells [1]. The signaling pathways regulating the development of MSCs are important in melanoma.

1.2 Vitiligo: Vitilgo is another skin disease related to depigmentation. It is a progressive skin disorder characterized by the loss of melanin. It is defined by depigmented, cutaneous lesions that develop following melanocyte death. While the condition is easier to detect in dark-skinned individuals, it afflicts people of all races. The melanocytes are the cells which produce melanin, which is responsible for the color of the skin. The microphthalmia-associated transcription factor (MITF) is the master regulator of melanocyte differentiation, development and survival and has been found to be closely associated with MITF.

A model has been proposed for the plausible role of MITF, dependent on different initiating stimuli, on the cell status. MITF regulation is governed by various genes/transcription factors and since, MITF is a transcription factor, it is involved in regulation of various other genes involved in signaling pathways. Several clinical observations suggest that there is a link between melanoma and vitiligo [18].

2. BRIEF LITERATURE:

2.1 Melanoma: Melanoma is a malignant tumor of melanocytes. Melanocytes are cells that produce the dark (brown-black) pigment, melanin, which is responsible for the color of skin (*Figure 1*). Melanin also helps to protect against the damaging rays of sun. When cells grow in a controlled manner, the resulting lesion is benign and is commonly referred to as a mole or nevus.

But when melanocytes grow out of control, they become malignant resulting melanoma. They predominantly occur in skin, but are also found in other parts of the body. Melanoma can originate in any part of the body that contains melanocytes.

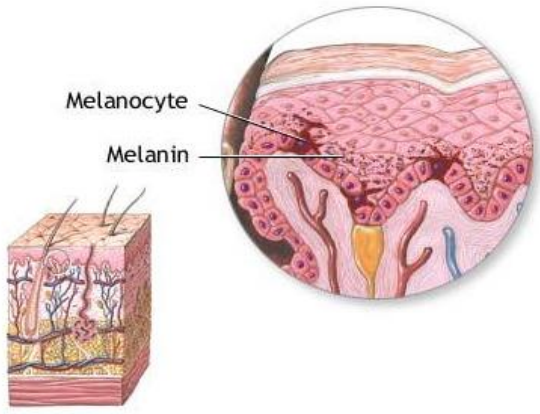

Figure 1: Melanocytes and melanin

2.2 MITF: The ***microphthalmia-associated transcription factor (MITF***) is the master regulator of melanocyte differentiation, development and survival. It plays a central role in the complex network of interacting genes regulating the migration, survival, proliferation and differentiation of melanocytes.

Because of its crucial importance in regulating the development of melanocytes, it is not surprising to find that at different stages of melanocyte development, MITF expression is regulated by an array of cooperating transcription factors that all influence how the MITF promoter responds to developmental signals.

MITF is also required to establish the MSC in the follicular niche and for this reason; it is thought to play a role in regulating extrafollicular dermal Melanocyte Stem Cells MSCs [1]. MITF is also effective in maintaining the tissue environment for development of normal cells [2]. A novel approach has been made to model and highlighted the role of MITF, dependent on different initiating stimuli, on the cell proliferation status. MITF regulation is governed by various genes/transcription factors and since, MITF is a transcription factor, it is involved in regulation of various other genes involved in signaling pathways.

2.3. Vitiligo: It is a skin disease characterized by white spots and patches. The white patch or spot is called as leucoderma and when it occurs without any preceding disease it is called Vitiligo*(Figure).*

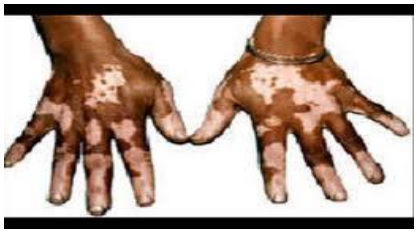

Figure 2. Skin of a vitiligo victim

Vitiligo is a disease which may be due to the presence of a situation of stress. This condition is triggered in genetically predisposed individuals, which destroys the melanocytes, showing the presence of an excess of free radicals in the areas of depigmentation phase when melanin becomes toxic to the cells that produced them. Taking antioxidant vitamins and minerals are highly recommended to enhance the treatment of vitiligo.

The toxins are accumulated in the liver and all those bodies responsible for their elimination, and this leads to remain undifferentiated melanocytes in the basal layer as a result of lack of blood flow, leading to the melanocytes lose their functions and remain in the basal cells as undifferentiated (i.e. no function to produce melanin which results in progressive depigmentation of the skin), and this coupled with the inability to acquire keratinocytes further enhances the severity of the disease. The UV radiations are also cause of this disease [3]. Imbalance of tissue oxidation-reduction system with production of free radicals is an initial pathogenic event in the degeneration of melanotic vitiligo [3].

The antioxidants have been useful in regulating the oxidative stress in this disease. Antioxidants are substances that act to protect cells from destruction by the chain reaction of electrons (cell death) caused by free radicals. Antioxidants are vitamins A, E, C, etc, and in minerals such as zinc, selenium and copper as well as compounds found in fruits and vegetables [3].

2.4 Gene regulatory network: *Gene regulatory networks* control changes in expression levels in response to environmental perturbations (*Figure 3)***.** The adaptation of cells to changes in their environment involves adjustment of gene expression levels.

A gene regulatory network or genetic regulatory network (GRN) is a collection of DNA segments in a cell which interact with each other indirectly (through their RNA and protein expression products) and with other substances in the cell, thereby governing the rates at which genes in the network are transcribed into mRNA. In general, each mRNA molecule goes on to make a specific protein (or set of proteins).

In some cases, this protein will be structural, and will accumulate at the cell membrane or within the cell to give it particular structural properties. In other cases, the protein will be an enzyme, i.e., a micro-machine that catalyses a certain reaction, such as the breakdown of a food source or toxin [4]. Some proteins though serve only to activate other genes, and these are the transcription factors that are the main players in regulatory networks or cascades. By binding to the promoter region at the start of other genes they turn them on, initiating the production of another protein, and so on. Some transcription factors are inhibitory.

In single-celled organisms, regulatory networks respond to the external environment, optimizing the cell at a given time for survival in this environment. Thus a yeast cell, finding itself in a sugar solution, will turn on genes to make enzymes that process the sugar to alcohol.

In multicellular animals the same principle has been put in the service of gene cascades that control body-shape [5]. Each time a cell divides, two cells result which, although they contain the same genome in full, can differ in which genes are turned on and making proteins. Sometimes a 'self-sustaining feedback loop' ensures that a cell maintains its identity and passes it on. Less understood is the mechanism of epigenetics by which chromatin modification may provide cellular memory by blocking or allowing transcription. A major feature of multicellular animals is the use of morphogen gradients, which in effect provide a positioning system that tells a cell where in the body it is, and hence what sort of cell to become. A gene that is turned on in one cell may make a product that leaves the cell and diffuses through adjacent cells, entering them and turning on genes only when it is present above a certain threshold level. These cells are thus induced into a new fate, and may even generate other morphogens that signal back to the original cell. Over longer distances morphogens may use the active process of signal transduction. Such signaling controls embryogenesis, the building of a body plan from scratch through a series of sequential steps. They also control maintain adult bodies through feedback processes, and the loss of such feedback because of a mutation can be responsible for the cell proliferation that is seen in cancer. In parallel with this process of building structure, the gene cascade turns on genes that make structural proteins that give each cell the physical properties it needs.

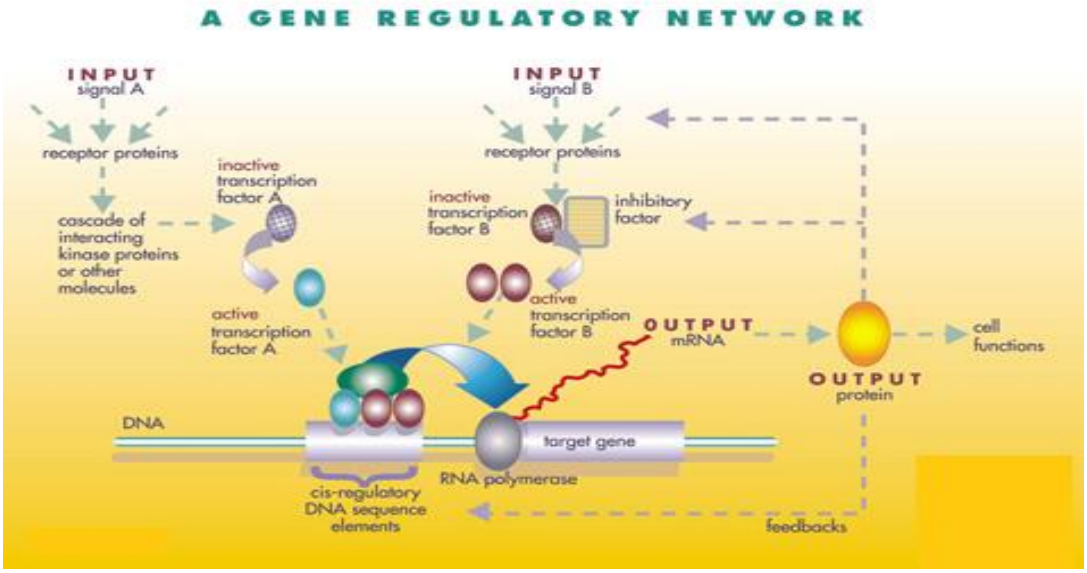

Figure 3. Gene regulatory network showing the role of transcription factors

It has been suggested that, because biological molecular interactions are intrinsically stochastic, gene networks are the result of cellular processes and not their cause (i.e. Cellular Darwinism). However, recent experimental evidence has favored the attractor view of cell fates

At one level, biological cells can be thought of as "partially mixed bags" of biological chemicals – in the discussion of gene regulatory networks, these chemicals are mostly the mRNAs and proteins that arise from gene expression. These mRNA and proteins interact with each other with various degrees of specificity. Some diffuse around the cell. Others are bound to cell membranes, interacting with molecules in the environment.

Still others pass through cell membranes and mediate long range signals to other cells in a multi-cellular organism. These molecules and their interactions comprise a *gene regulatory network (Figure4)*. A typical gene regulatory network looks something like this:

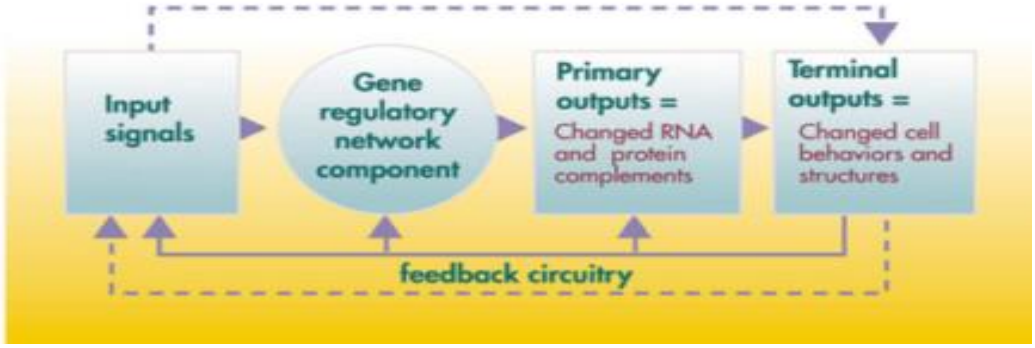

Figure 4. Feedback network effecting the gene regulation and cell behaviour

The nodes of this network are proteins, their corresponding mRNAs, and protein/protein complexes. Few nodes are associated with the cell/environment interfaces, while the others are free-floating and diffusible. Implied are genes, the DNA sequences which are transcribed into the mRNAs that translate into proteins. Edges between nodes represent individual molecular reactions, the protein/protein and protein/mRNA interactions through which the products of one gene affect those of another, *though the lack of experimentally obtained information often implies that some reactions are not modeled at such a fine level of detail*. These

interactions can be inductive, with an increase in the concentration of one leading to an increase in the other, or inhibitory, with an increase in one leading to a decrease in the other.

A series of edges indicates a chain of such dependences, with cycles corresponding to feedback loops. The network structure is an abstraction of the system's chemical dynamics, describing the manifold ways in which one substance affects all the others to which it is connected. In practice, such GRNs are inferred from the biological literature on a given system and represent a distillation of the collective knowledge about a set of related biochemical reactions.

Genes can be viewed as nodes in the network, with input being proteins such as transcription factors, and outputs being the level of gene expression. The node itself can also be viewed as a function which can be obtained by combining basic functions upon the inputs. These functions have been interpreted as performing a kind of information processing within the cell, which determines cellular behavior. The basic drivers within cells are concentrations of some proteins, which determine both spatial (location within the cell or tissue) and temporal (cell cycle or developmental stage) coordinates of the cell, as a kind of "cellular memory". The gene networks are only beginning to be understood, and it is a next step for biology to attempt to deduce the functions for each gene "node", to help understand the behavior of the system in increasing levels of complexity, from gene to signaling pathway, cell or tissue level.

Mathematical modeling of GRNs have been developed to capture the behavior of the system being modeled, and in some cases generate predictions corresponding with experimental observations. *In some other cases, models have proven to make accurate novel predictions, which can be tested experimentally, thus suggesting new approaches to explore in an experiment that sometimes wouldn't be considered in the design of the protocol of an experimental laboratory.* Taken together, coupling and coordination of genes is responsible for its signaling pathway and subsequent target gene expression.

2.5 Effect of MITF on p21/p16 :

Mitf can act as an anti-proliferative agent by activation of $p21^{Cip1}$, CDKN1A cyclin dependent kinase inhibitor gene [6]. Mitf interacts with pRb and this cooperation increases the ability of Mitf to activate p21 [6,7]. p21 is a universal inhibitor of cyclin kinases which controls the cell cycle progression in G1 and S phase. Expression of p21 is generally regulated by p53 but in some cases it can be expressed without being induced by p53. Significantly, $p16^{INK4}$ expression in melanocytes is associated with increased level of MITF and it regulates the cell cycle exit by activating the cell cycle inhibitor INK4A in normal melanocytes[8]. p16, along with p14, functions as a tumor suppressor [7]. p21 can mediate cellular senescence and one of the ways it was discovered was as a senescent cell-derived inhibitor. The p21(CIP1/WAF1) protein can also interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP-3 like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation.

2.6 Effect of MITF on B-RAF:

The mutations of genes like BRAF could affect either the stability of MITF or its cooperation with pRb [6]. B-RAF is an oncogene which is mutated in most of the human melanomas and transformed mouse melanocytes.

Mitf expression is suppressed by B-RAF in immortalized mouse and human melanocytes. MITF reexpression in BRAF transformed melanocytes inhibits their proliferation. It is found that differentiation-inducing factors that elevate MITF expression in melanoma cells inhibit their proliferation, but when MITF up-regulation is prevented by RNA interference, proliferation is not inhibited [9]. This

is again an evidence of anti-proliferative action of MITF under regulation and influence of certain differentiating factors. Melanocytes expressing ^{V600E}BRAF display reduced dendricity and pigmentation (which is against the impact of MITF in melanocytes) and MITF was consistently down-regulated in cell lines expressing ^{V600E}BRAF which correlates with the activation of ERK activation. ATRA has been shown to suppress BRAF/ERK signaling in mouse skin cancer model [10]. It has also been shown that senescence mediated by c-Myc suppression in melanoma is p53 independent but relies on ERK pathway activation [11]. Moreover, c-Myc suppression increases the level of p21, which is activated by MITF and this ability of MITF is aided by retinoid dependent genes.

Microphthalmia-associated transcription factor (MITF) is associated with the regulation of many factors related to melanocyte survival and differentiation. It controls the transcription of genes involved in cell cycle progression, cell survival, migration and angiogenesis. MITF has been implicated in both, proliferative as well as anti-proliferative activity of normal cells and melanoma cells.

A model proposed by Carriera et.al. has been shown in *Figure 5.* In this model, high MITF level is associated with differentiation and lower level with proliferation. Transient MITF silencing is with quiescence and stem cell phenotype, whereas sustained MITF silencing has been shown to trigger cellular senescence

It has been shown that deletion of MITF is sufficient to increase the metastatic potential of mouse and human melanoma cells[12] MITF level decreases during melanoma progression and diminished MITF expression is associated by less differentiation, which may provide growth advantage in melanoma [13]Also, MITF forced expression decreases tumor and metastasis formation [12].

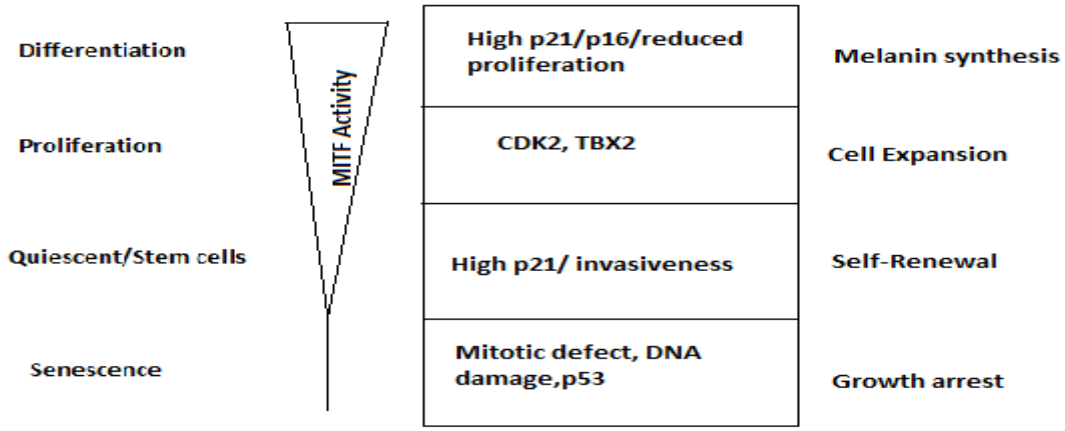

Figure 5: MITF rheostat model by Carreira et. al.2005

High MITF level is associated with differentiation and lower level with proliferation. Transient MITF silencing has been associated with quiescence and stem cell phenotype, whereas sustained MITF silencing has been shown to trigger cellular senescence.

3. METHODOLOGY:

A *homeostatic* biofeedback model comprising of brain and endocrine regulator and their consequent *transduction phases* has been realized by MATLAB (Simulink) tool.

The input stimulus is taken as a step function because any other form of input could be simplified in the form of step function [14] The homeostatic transfer functions are taken as first order systems for the sake of simplicity, although it could be a higher order term. The per unit scale values signify normalization of the curve to correlate a particular physiological phenomenon. The output has been simulated in MATLAB (7.6, R2008a) SIMULINK.

Per unit values are taken as maximum expression level of genes. The exponentially varying curves are obtained after implementing the second and first order transfer functions and the coupling effect to the input signal. The two different outputs are obtained by varying coupling factors and loss components of the coupler block.

4. MODELING:

The modeling has been done in MATLAB SIMULINK environment, taking MITF into consideration and the antioxidant as input as for decreased metastasis, and carcinogenic stimuli for increased metastatic effect of various target genes.

The present model comprises of input as step function because all other forms of input could be simplified in the form of step function. The input is governed by brain and endocrine regulator, which are responsible for directing the signaling pathways. It consists of closed loop comprising of MITF homeostat and its corresponding transduction phase, thereby affecting the activity of MITF. The modified MITF output is taken as the input for the next stage and also effects the related genes/proteins as discussed above. Hence, this output effects the status of retinoblastoma phosphorylation along with other relevant genes/proteins specified above. The next stage of the model is p21 homeostat, which along with the other relevant genes, affects the overall response of the system. The next stage considers the coupling of these modified genes related with the input signal.

The transfer function of MITF homeostat is taken as

$G1(s) = 5/(s+2)$ and transfer function of corresponding transduction phase is taken as

$H1(s) = 0.2/(s^2 +s +1)$

such that the overall transfer function becomes

$T1(s) = G1(s)/(1+ G1(s)H1(s))$

$=5(s^2+s+1)/(s^3+3s^2+3s+2)$

The homeostat is taken as first order system for simplicity, although it can be of higher order in real system. The output1 gives output as MITF level which is associated with phosphorylation status of pRb and related genes like ATF2.

This output acts as input to second stage comprising of p21 homeostat.

The homeostat2 (p21) has transfer function of

$G1(s) = 5/(s+5)$

and transfer function of corresponding transduction phase is taken as

$H1(s) = 0.1/(s^2 +s +1)$,

such that the overall transfer function becomes

$T1(s) = G1(s)/(1+ G1(s)H1(s))$

$=5(s^2+s+1)/(s^3+6s^2+6s+5.5)$

This output acts as input to the third stage comprising of the coupler. It is worthy to note that only the prescribed genes are not responsible for the complete process of differentiation and growth arrest; there are many more factors responsible for MITF status. The first stage block diagram representation is shown in *Figure 6a and 6b.*

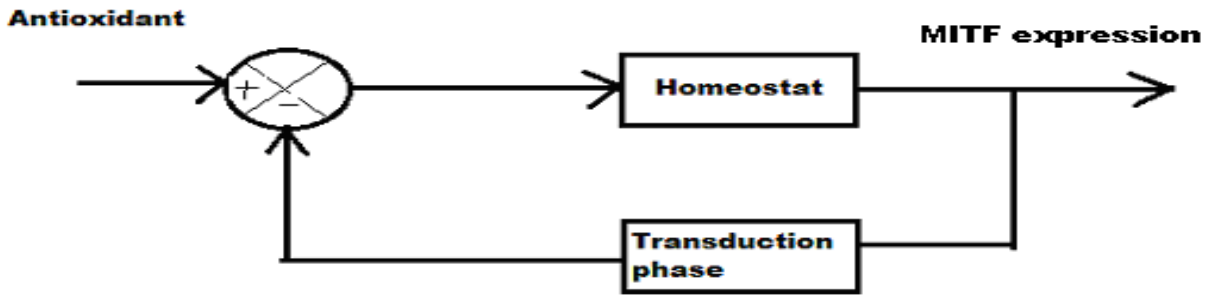

Figure 6a: MITF output corresponding to antioxidant stimulus

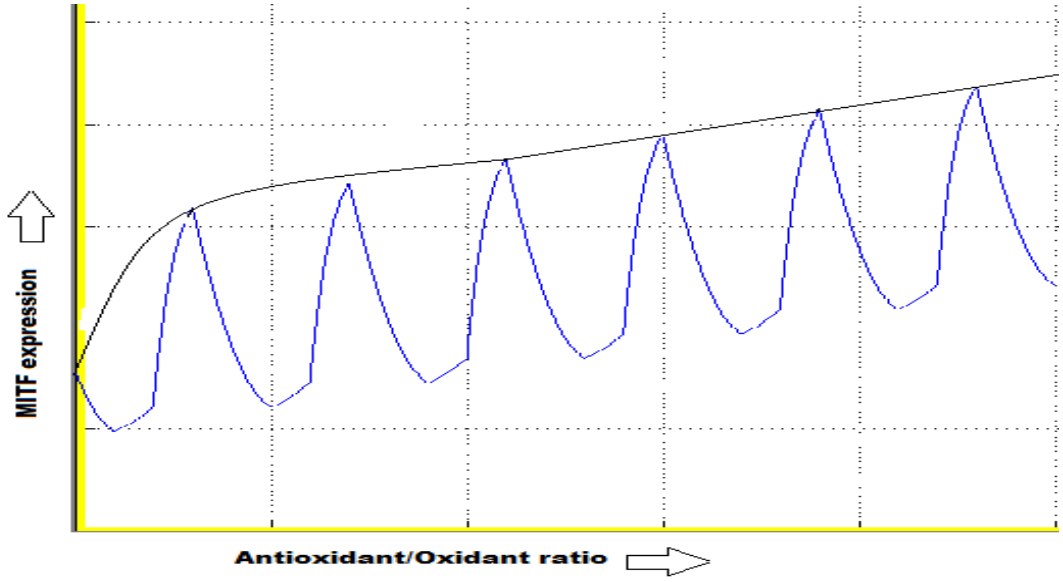

Figure 6b: MITF output corresponding to antioxidant stimulus

Taking these factors together the MITF model *(Figure 7)* has been proposed with two threshold levels and four layers. The shaded layer represents the safe MITF level which regulates the normal cell cycle. The increased MITF expression above the normal level resembles the melanoma stage. If the MITF level is increased above the upper threshold, the proliferation is reduced and improves the status of melanoma. The downregulation of MITF below normal level resembles vitiligo. The upregulation of MITF in vitiligo tends to move toward normal status and further downregulation tends to senescence.

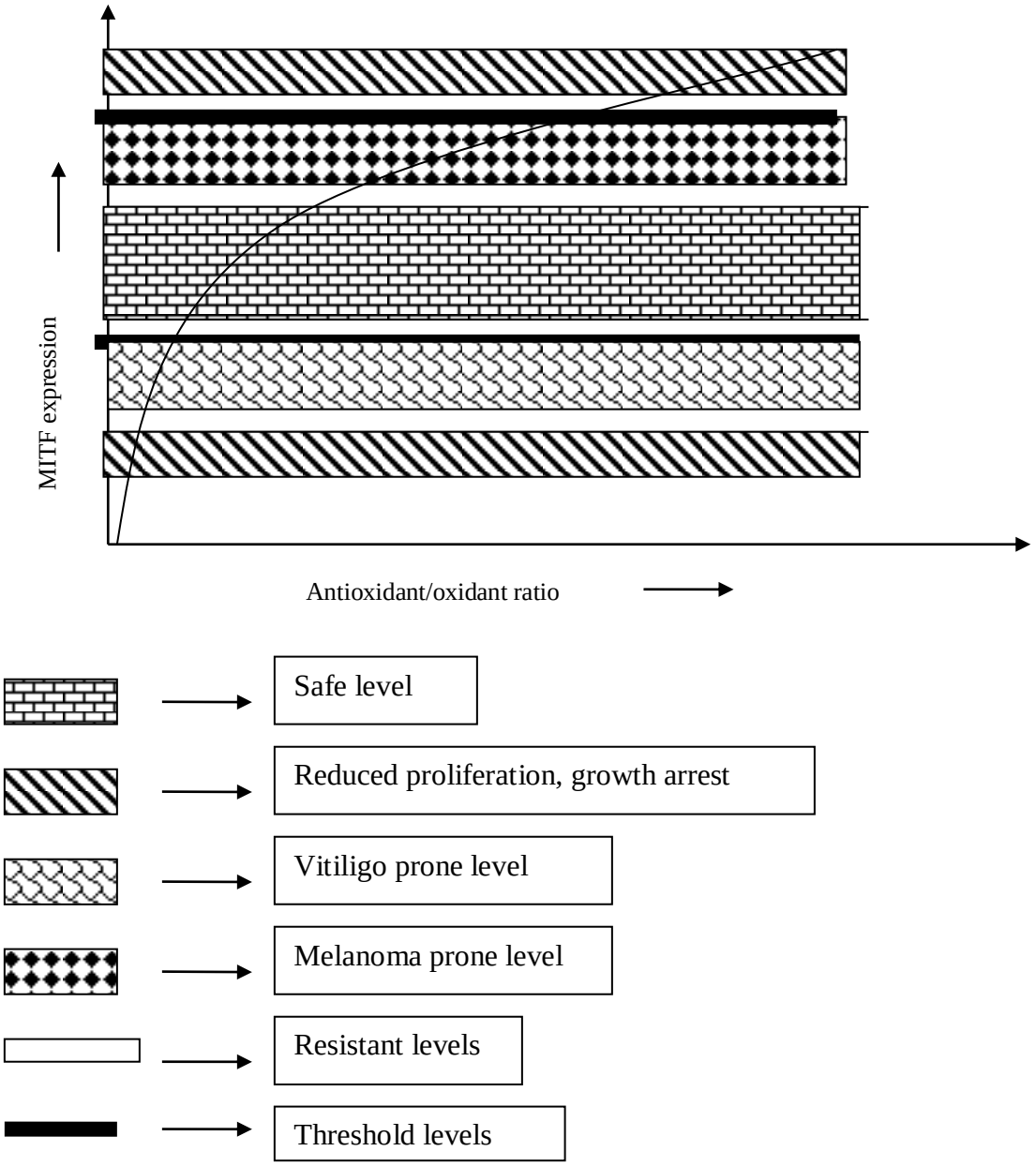

Figure7: MITF activity model corresponding to antioxidant stimulus

4. DISCUSSION:

The model shows that the MITF expression is improved with antioxidant intake which is better for both melanoma and vitiligo. A survey suggests that the risk of melanoma decreases in patients with vitiligo [15]. Moreover, vitiligo has been a positive prognostic factor for melanoma patients. It is found that there is upregulation of p53 in vitiligo patients [16]. Now p53 can regulate cell cycle arrest via p21[17] and p21 is modulated by MITF. MITF is downregulated by oxidative stress in the epidermal cells of normal and vitiligo effected cells [17]. Hence, according to this model, the decreased risk of melanoma in vitiligo patients is owing to the resistance offered by two threshold layers which has to be overcome to trigger melanoma.

5. CONCLUSION:

The alteration in MITF target is effected by antioxidant and regulates the homeostatic response of associated gene/s which is known markers of cell cycle regulation. The model has related the MITF level with other antioxidant dependent gene/s, some of which are

tumor suppressive genes. Since, MITF is a transcription factor; it affects the signaling pathway of normal and malignant cells. The model reveals the importance of coupling of MITF with antioxidant dependent genes in melanoma.

The MITF level has to be increased above threshold value for growth arrest in melanoma. The capability of increasing MITF above this threshold value also depends on the initial microenvironment of the individual cell and tissue. Any discrepancy in fulfilling this condition may alter the MITF mediated signaling pathway.

Interestingly, MITF silencing is also associated with apoptotic effect. But MITF is also associated with normal function of melanocytes and melanin formation. This gives an explanation of occurrence of vitiligo in melanoma patients undergoing melanoma treatment.

The above results and explanations imply that the regulation of MITF is a very important factor in cell cycle regulation. Although, MITF is regulated by many factors, a common factor which induces MITF and increases its expression for preferring cell differentiation is the oxidative microenvironment of the related cell and tissue. The oxidative stress in cells is a common factor in both, melanoma and vitiligo and both these diseases are associated with MITF expression. Although, the important and active role of MITF has been described, the potential role of MITF in these two diseases further needs to be clinically proven.

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