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Immunomics in Skin Cancer - Improvement in Diagnosis, Prognosis and Therapy Monitoring

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Abstract: This review will focus on the elements of the skin's immune system, immune cells and/or non-immune cells that support immune mechanisms, molecules with immune origin and/or immune functions that are involved in skin carcinogenesis. All these immune elements are compulsory in the development of skin tumors and/or sustainability of the neoplastic process. In this light, recent data gathered in this review will acknowledge all immune elements that contribute to skin tumorigenesis; moreover, they can serve as immune biomarkers. These immune markers can contribute to the diagnostic improvement, prognosis forecast, therapy monitoring, and even personalized therapeutical approach in skin cancer. Immune processes that sustain tumorigenesis in non-melanoma and melanoma skin cancers are described in the framework of recent data.

Keywords: Basal cell carcinoma, immunomics, lymphoma, melanoma, squamous cell carcinoma.

INTRODUCTION

Immunomics Reloaded

The "Omics" vision has been lately extended to the immune system. Immunomics, an integrated approach for scrutinizing the immunome, seeks to explain the entire immunological process of an organism starting with antigen recognition and ending with the development of a requested immune reaction elicited by the antigens presented to the immune system [1, 2]. As other *–omics*, immunomics is a dynamic discipline relying on highly specialized computational approaches and various immunoassay techniques in order to catalogue and predict the epitopes able to elicit an immune response [3].

Recently published, the concept of *Tumor ImmunoEnvironment* sheds a new light on the complex mechanisms that underlie the cellular struggle of malignant cells and immune elements in the architectural context of skin tissue. In this concept, the immune cells resident in the tissue and/or circulating throughout the lymph system coordinate cellular events in tumor tissue microenvironment. There is a subtle equilibrium between anti-tumoral immune cells and those that favor tumor progression. Thus, the tumor microenvironment provides various factors of malignant and non-malignant origin that can guide anti-tumoral immune surveillance or favors their development, owing to immune escape mechanisms.

This new concept explains the tumor microenvironment guiding tumor development stages. Processes such as inflammation and tumor-mediated immunomodulation govern cellular interactions in a step-by-step evolution of cancer, mechanisms that are involved additionally in the efficient therapeutical response [4].

"Omics" technologies that have flourished in the last decade tackle multiple molecules like the entire genome, transcriptome, proteome, and so on. For several reasons, one of the first organs subjected to proteomics/genomics was skin. Skin is readily accessible, it can provide large samples, has intricate cellular populations comprising resident immune cells and non-immune cells and, last but not least, it develops a complex array of pathologies. Among these pathologies, skin cancer with all its variants, melanoma and non-melanoma, offered proteomics a solid foundation for biomarker discovery in diagnosis, prognosis and therapy monitoring.

Searching the skin's proteome, the keratinocyte came into the spotlight. A much ignored cell, epidermal keratinocytes were shown to respond to inflammatory and immunomodulating cytokines, in addition to chemical and physical factors. As physical barrier of the organism, skin is continuously subjected to the environment, and keeping this homeostasis requires harboring complex immune elements. In the field of skin's tumorigenesis, dermato-oncology has taken *omics* domain through important steps to personalized medicine [5].

SKIN'S IMMUNE SYSTEM

The skin immune system (SIS), the largest immune organ [6] is comprised of a complex network of humoral and cellular immune effectors to fight against biological, physical, or chemical assaults [7]. Cell populations that cooperate to develop the cellular components of SIS include keratinocytes, dendritic antigen-presenting cells (APCs), monocytes/macrophages, granulocytes, mast cells, lymphatic/vascular

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endothelial cells, and T lymphocytes [8]. This array of cell populations, of immune and non-immune origin, intercommunicate *via* secreted molecules such as cytokines, chemokines, neuropeptides, eicosanoids, prostaglandins, free radicals, antimicrobial peptides, complement components, immunoglobulins, fibrinolysins, and so on [7]. The specific immune arms of SIS are lymphocytes that extravasate from circulation into the skin [9]. Presenting antigen cells follow a clear route to and from the skin and lymph nodes, with the route patrolled by Langerhans cells (LCs) and dermal APCs. Keratinocytes and endothelial cells, non-immune cells by definition, produce immune-related molecules, such as cytokines and growth regulatory factors. All these elements interplay, in order to achieve skin immune surveillance [10], playing a crucial role in maintaining skin's homeostasis. Cellular components of SIS comprise B (when skin is aggressed) and T lymphocytes, members of the adaptive immune
system, while innate immunity is represented by APCs, mononuclear phagocytes and dendritic cells (DCs). The innate arm response is rapid, but nonspecific, while the adaptive arm refines the immune response, it is specific, slower, and elicits long-term memory [10].

The keratinocytes, the non-immune cells involved in the development of the skin's immune response, cooperate with T lymphocytes secreting immune response-eliciting cytokines. It has recently been found that co-culturing keratinocytes with T cells, keratinocytes pushed T subpopulations toward specific phenotypes such as IL-17+CCR6+RORγt+ (Retinoic acid-related Orphan Receptor γT). Moreover, T lymphocytes isolated from skin are in part IL-17+, confirming that there is a clear intercellular communication between immune T cells and non-immune keratinocyte cells and that any deregulation in the information circulation can trigger skin disease [11]. Keratinocytes, as demonstrated 20 years ago, secrete large quantities of interleukin-1alpha (IL-1alpha), tumor necrosis factor (TNF) and neuropeptides in response to various stimuli (kinetic, thermal trauma, UVB irradiation) [12]. IL-1alpha induces lymphocytes and neutrophils migration *via* the increased expression of CCL20 on keratinocytes [13]. In summary, IL-1alpha (and IL-1beta secreted by epidermal LC) is a potent stimulator of local immune function. As one of the key immunoregulatory cytokines, IL-1alpha corroborates with several immune-related molecules modulating the skin resident innate immune cells, mast cells, DCs, and macrophages that will recruit additional immune cells from the circulation [14].

The other non-immune cells, extremely important in the proper functioning of SIS, are the microvascular endothelial cells [6]. Looked upon as having a ''passive'' role, these cells are the actual crossways where leukocytes extravasate. Endothelial cells link the dermal compartment to the intravascular one, mediating the transport of chemotactic and stimulatory signals. Furthermore, cytokines like TNFalpha induce the increment of adhesion molecules expression on vascular endothelium, leading to the production of chemo attractants that will attract leukocytes into the skin [15]. Recent results in experimental models have shown that endothelial cells acquire the antigen (Ag) and present it to the localized T lymphocytes; T cells become activated, produce cytokines and trigger the initiation of innate immune response [16]. Thus, endothelial cells take the function of APCs to intravascular T cells.

In the epidermis, the resident immune cells are specialized DCs (LCs) and intraepithelial T lymphocytes. In normal dermis, dermal DCs, mast cells, and a small number of CLA+ memory T cells are the resident immune cells in this compartment. The dermal post-capillary venule is an *immune avenue* having a basal adhesion molecules expression. Thus, these venules constitutively express low levels of E-selectin, CC-chemokine ligand 17, and ICAM-1, all of which guide the circulation and margination of CLA+memory T lymphocytes into non-aggressed skin. The adhesion molecules expressed by the dermal post-capillary venules actually select the cells that will remain in the skin and transmigrate. Cells like CLA- T lymphocytes, naive cells or memory/effector cells that are prone to other tissues, granulocytes and other immune cells that do not have appropriate receptors to attach to dermal vessels, will not enter the skin's layers and will not contribute to SIS [8]. Taking into account that skin is the largest organ with immune function, this clear cell selection of immune cells that comprise resident SIS represents a mandatory process for proper homeostasis.

The concept of skin immune surveillance, stated approximately 15 years ago, is a complex mechanism. Immune surveillance has three, rather didactically ranked, stages. The primary response initiates the engagement of the adaptive immune response in which Ags encountered in the skin are carried by activated DCs and presented to naïve and central memory T cells circulating through the lymph node. T cells that encounter the Ag proliferate and differentiate into effector cells expressing homing receptors. The secondary stage in the skin immune surveillance concept ensures rapid and effective local adaptive immune responses to previously encountered Ags, up regulating the expression of adhesion molecules and presentation of specific chemokines on the local endothelium. Effector memory T cells are recruited in an Ag nonspecific manner. The last type of skin immune surveillance, the tertiary, enhances adaptive immune responses to Ags encountered in tissues distinct from those in which they were previously encountered. Central memory T cells produced in skin draining lymph nodes recirculate through lymph nodes throughout the body, providing enhanced responses to Ag that were previously encountered in a different tissue [17].

In all of these surveillance levels immune-markers can be found, whether cells or immune intercommunicating molecules, that indicate the triggering of an immune response [18].

Beside resident immune cells, the first line of defense, there are constant populations of circulating immune cells recruited in the site of injury. This circulation is sustained by an array of chemokines and chemokine receptors that regulate this migration of immune cells. A recent review showed that CCR10 (chemokine receptor) and its ligands, CCL27 and CCL28, sustain this immune migration. Thus, ligand CCL27 is expressed by keratinocytes, while ligand CCL28 is expressed by mucosal tissues epithelial cells. Their receptor CCR10 is expressed by various subsets of T

lymphocytes that are likely to come to the skin during their thymic development. If a local immune response is needed, circulating T cells can be recruited to the skin by APCs that induce on their surface CCR10. This molecule's tandem, chemokine receptor CCR10 and its ligands can be "hijacked" by cancer cells and used for their proliferation and evasion from immune surveillance [19]. Recent experimental data showed that, when culturing human keratinocytes directly with T lymphocytes (CD4+ or CD8+) chemokines like CCL2, CCL20 and CXCL10 are actively secreted [11].

In a recent experimental model, the gradient of CCL21 was proven to conduct DCs in the lymphatic circulation. The haptotaxis process is explained by the CCL21 being immobilized to heparan sulfates, inducing migration of immune cells along immobilized gradients in tissues [20].

IMMUNE MECHANISMS OF SKIN TUMORIGENESIS

SIS has as main role the development of anti-tumoral surveillance mechanisms; however, when it is overrun, it can sustain tumorigenesis. For skin, UV irradiation is one of the main triggers of tumorigenesis. In the early phases of UV-irradiation, SIS sustains the immune protection. The skin reacts to all damages, whether physical injuries or biological, therefore UV-irradiation triggers immediate innate immunity and, if needed, afterward develops the more specific acquired immunity. Increased secretion of defensins and complement pro-inflammatory mediators triggers the further specific acquired immunity. On the other hand, all inflammatory triggered mechanisms can accelerate the tumorigenesis processes by increasing all immune-type molecules and cells that sustain immunosuppression and skin cancer development [21]. One of the cells that have been newly reported as suppressing the UV-triggered anti-tumor immune response is the mast cell. Known as a cell type involved in allergies, the dermal mast cell was shown to secrete, upon UV-irradiation, skin tissue remodeling and pro-angiogenic factors that can promote skin cancer processes [22].

Unfortunately, SIS contributes to the tumorigenesis process, with specific adhesion molecules and nitric oxide (NO), a highly immune-related molecule. As stated in the prior section, endothelial cells are important non-immune SIS cells. In a recent study that aimed to establish the involvement of these cells in skin tumorigenesis, the role of NO in inducing endothelial cell permeability and leukocyte transmigration was studied. In melanoma skin cancer progression, NO down-regulation induced a decrease in endothelial cell ability to adhere, a process developed by the inhibition of PECAM-1/CD31 expression. Moreover, NO is involved in the modulation of the expression of several adhesion molecules CD34, ICAM-1/2 and VCAM-1. This process affects the leukocyte adhesion on endothelial cells. The authors show that NO is involved in angiogenesis, leukocyte transmigration, and therefore in skin tumorigenesis [23].

Altering LC's structure and function - alteration due to UVB irradiation and/or chemical carcinogens - can induce by itself the disruption of the local immune response and tolerance generation that favors the development of neoplastic cells [24].

In a recently published study performed on animals, mouse tumor-infiltrating granulocytic and monocytic populations, myeloid-derived suppressor cells (MO-MDSCs) were isolated from experimental melanoma tumors. The populations infiltrating the tumors showed high levels of chemokines ligands CCL3, CCL4, and CCL5. Receptor CCR5 was found on regulatory T lymphocytes (Tregs). The authors found that *in vitro* MO-MDSCs expressing high levels of CCR5 attracted increased numbers of Tregs, maintaining immune-suppressions. When directly inoculating intratumoral CCL4 or CCL5, an increased number of Tregs infiltrated the tumor, while the lack of CCR5 abolished Treg's infiltration. This recent experimental data demonstrates that myeloid-derived suppressor cells secrete chemokines that induce a local immune suppression by attracting large numbers of Tregs [25].

Lymphocytes that have Tregs phenotype/genotype are extremely important in skin tumorigenesis. The costimulatory molecule CD28 is a crucial molecule for naive T cells activating and overall functioning. In a Treg-specific CD28 conditional knockout mice it was recently published that, although T cells were FOXP3+, the large numbers of activated T cells induced autoimmunity in skin and lungs. In this model, Tregs lacked CTLA-4, PD-1, and CCR6 expression. The authors point out that CD28 in FOXP3+ Tregs induces complete functioning and moreover, the established role of CD28 can be exploited in skin tumorigenesis [26].

Skin inflammation is one of the processes that has a dual role, as it can favor tumorigenesis or it can trigger anti-tumoral immune actions. Recently, a proinflammatory cytokine, thymic stromal lymphopoietin (TSLP), has been studied for its involvement in skin carcinogenesis. In an experimental mouse model, it was shown that TSLP and the induced inflammation acts on CD4 and CD8 T lymphocytes, inhibiting skin carcinogenesis. When its specific receptor, TSLP receptor (TSLPR), is genetically knocked-out, this anti-carcinogenesis effect is lost. Myeloid suppressor cells accumulate, and tumor growth is accelerated *via* Wnt ligands and β-catenin signaling pathways. One of the recent published studies proves this dual role of inflammation in skin carcinogenesis, both pro and anti-tumorigenic [27]. In the same light, also recently reported, endogenous molecules named alarmins can induce an efficient immune response or can hinder its anti-tumoral activity. An enhanced release of alarmins from the damaged tissue can contribute to skin tumorigenesis and metastasis. In the same way, alarmins can contribute to tissue homeostasis, repair, remodeling in several types of tissues including skin [28].

One of the recently involved immune-related molecule in tumorigenesis, mediating as well inflammation, is interleukin-17 (IL-17), secreted by activated T lymphocytes. It was first characterized approximately 10 years ago and since then it has expanded to a complete family of IL-17 cytokines. The first discovered member was IL-17A, and due to genomic and proteomics continuous development, new members joined the family, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. These cytokines link to specific receptors: IL-17R, IL-17RH1, IL-17RL (receptor like), IL-17RD and IL-17RE. Among several organs and tissues, IL-17 is active in skin tumorigenesis [29]. An experimental mouse model has

recently been published for skin cancer, identifying the mechanisms for IL-17 involvement in tumorigenesis. Mice deficient in IL-17 receptor-A gene (IL-17R-/-) were insensitive to chemical carcinogen-induced skin carcinogenesis. It is known that inflammation induces skin hyperplasia. Production of cytokines with pro-tumor inflammatory function molecules was decreased in mice with IL-17R-/- genotype. In these mice, cells that sustain effector functions, CD8+, have an increased percentage in TIL, while CD11b+ myeloid cells with suppressor functions are decreased. Skin inflammation favors tumor development and appearance of tumor specific IL-17 secreting T lymphocytes. Overall, the authors report that IL-17 controls inflammation and sustains tumorigenesis. The cytokine IL-17 becomes an interesting future immune-marker and potentially a future therapy target [30]. The family of IL-17 and their receptors are involved in skin homeostasis in SIS and can furbish future therapy targets in skin cancer.

Non-melanoma skin cancer has as subtypes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). These subtypes have different characteristics, both clinical and immune-related. An exhaustive proteomic tissue microarray study of these types of skin cancer revealed the protein expression profiles of BCC and SCC. Authors correlated their proteomic data with pre-cancerous stages like actinic keratosis, Bowen's disease, seborrheic keratosis and normal epidermis. They have reported several protein changes in these diseases in terms of proteins involved in cell interactions, cell growth, cell cycle regulation or apoptosis. Among several proteins that have been found to be decreased, including collagen XVII, CD44v6, pan-Desmoglein, the expression of adhesion molecule E-cadherin was found decreased. Finding this adhesion molecule differently expressed in BCC compared to SCC, the invasiveness difference was also acknowledged in BCC compared to SCC [31].

Epidermal growth factor receptor (EGFR) is known as a crucial molecule for tissue development. Recently, it was demonstrated that in inflammation, Tregs express EGFR. Mast cells secrete an EGF-like growth factor, named amphiregulin (AREG) and thus increase Treg functions. Hence, these recent data show that EGFR is a non-immune molecule regulating immune functions [32]. EGFR was found highly expressed in SCC compared to BCC, and this finding can additionally account for the lower invasiveness of BCC compared to SCC. Proteins can account for the stated different immunobiology of these non-melanoma skin cancers [31].

Basal Cell Carcinoma

One of the most frequent types of non-melanocytic tumors is BCC, associated with a cellular infiltrate rich in immune cells as other skin cancers. More than 30 years ago, the group of Dellon *et al.* [33] was the first to publish the existence of deregulations in the peripheral immune populations of patients diagnosed with BCC. In these patients, they found a significantly lower number of circulating T lymphocytes compared to controls, and that the decrease of circulating immune cell populations correlated with tumor size. After another 15 years, the group of Myskowski *et al.*, observed that the activation properties of immune cells, proliferation response to a mitogen, like concanavalin-A is reduced in BCC patients [34]. Then, in the 1990's, along with T cell subsets reduction, NK cells impairment was reported [35]. So, more than 20 years ago, it was shown that BCC infiltrate consists mainly of T lymphocytes, LC are often present around the tumor nests, while NK or B cells in the infiltrate are very rare [36]. T subpopulations, both CD4+ and CD8+, are present in the tumor infiltrate in a CD4/CD8 ratio of around two [37]. Most peritumoral inflammatory cells have an activated phenotype, HLA-DR+, CD25+, CD45RO + and so on. However, BCC tumor cells are HLA-DR-, ICAM-l-, and express low levels of MHC class I. This phenotype of the BCC tumor cell makes it somewhat "invisible" to the immune effectors. When BCC regress spontaneously, a significantly increased number of CD3+ CD4+ T lymphocytes can be identified in the regressed tumors.

Treg FOXP3+ cells were also identified in BCC, being involved in rejection of tumors induced by UV irradiations. Suppression of efficient immune response by Treg is sustained by IL-10 and TGFb secretion. These cytokines compete with effector T lymphocytes for IL-2 and inhibit the antigen presentation by DC [38]. An increased number of Treg in BCC is associated with a poor outcome. However, a comparison of the number of Tregs with other T lymphocytes subsets is difficult to do because the FOXP3 marker could be transiently expressed on other T activated cells as well. Activation and proliferation of T cells starts with antigen recognition by DC. We must take into account that BCC cellular infiltrates have high numbers of immature myeloid DCs CD11c+, thus creating an immune suppression milieu. Immature DC can induce T lymphocytes functional anergy [39]. The presence of tumor associated macrophage (TAM) populations in the BCC infiltrate is correlated with an increased expression of COX-2, resulting in tumor invasiveness and growth of microvascular density [40].

There are several immune mechanisms for the evasion of immune responses by BCC tumors (Fig. **1**). As previously stated, inconsistent expression of HLA-ABC (MHC l) and/or complete lack of HLA-DR on BCC cells makes them unrecognizable to the SIS. The absence of any co-stimulatory molecules, such as ICAM-1, CD40 and CD80, on BCC cells can induce T cell anergy when TCR-MHC interaction occurs. Tumor BCC cells can produce IL-l0, a cytokine that can account for the lack of HLA-DR, ICAM-1, CD40 and CD80, for the inconsistent expression of HLA-ABC on BCC cells, and for the induction of T cell anergy. IL-10, moreover, reduces the production of pro-inflammatory cytokines, such as IFN-gamma, by T cells and the down-regulation of IFN-gamma specific receptors on the BCC tumor cells. Shedding of ICAM-1 in the environment can trap T lymphocytes and hinder an eventual tumor cell-T interaction [41].

When therapy is applied in BCC, one can observe the re-equilibration of immune mechanisms. Using imiquimod, the effector response *via* CD3+/CD4+ T lymphocytes is increased, along with a slight increase in NK cells and Tc CD3+/CD8+. Treatment can lead to CD20+ B cells increment in the lesional site and a slight increase in monocyte-macrophages with a CD68+ phenotype. Upon treatment LC, CD1A+, were found decreased in the epidermis while

DC with a CD1A+ phenotype were found increased. The authors ascertain that BCC regression has a clear pro-inflammatory effect sustained by CD3+/CD4+ T lymphocytes and a pro-apoptotic activity *via* decreased Bcl-2 expression [37].

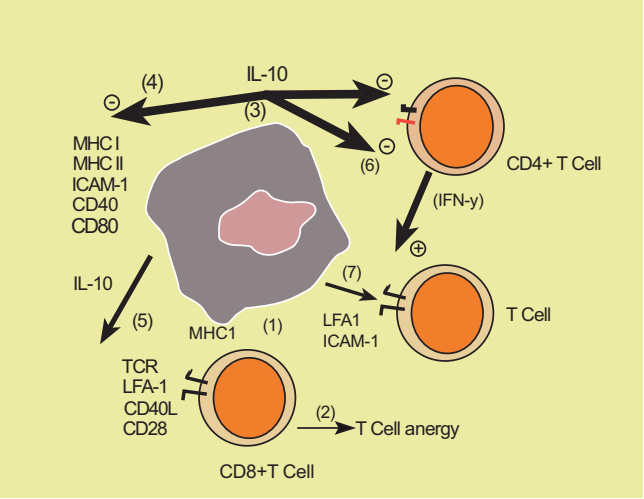

Fig. (1). Immune mechanisms that sustain BCC tumorigenesis.
There are several mechanisms that conjoin in skin tumorigenesis in BCC: (1) inconsistent expression of HLA-ABC (MHC l), complete lack of HLA-DR on BCC. (2) absence of costimulatory molecules, ICAM-1, CD40 and CD80, on BCC cells that can induce T cell anergy when TCR-MHC interaction occurs. (3) BCC cells can produce IL-l0, cytokine that can account for the lack of HLA-DR, ICAM-1, CD40 and CD80 and the inconsistent expression of HLA-ABC (4) on BCC cells and the induction of T cell anergy (5). IL-10 moreover reduces the production of pro-inflammatory cytokines, such as IFN-gamma by T cells (6) and the down-regulation of IFN-gamma specific receptors on the BCC tumor cells. (7). Shedding ICAM-1 in the environment can trap T lymphocytes and hinder an eventual tumor cell-T interaction (adapted from [41]).

Basal cell carcinoma represents the most common skin cancer, and SIS is highly involved in development and further progression of the tumor; the development of an anti-tumor-specific immune response can be altered by subtle mechanisms and even by stress [42]. The specific role of immune cell populations in BCC, as in other non-melanoma skin cancers, continues to be explored in search of new biomarkers and new immune networks.

Squamous cell Carcinoma

SCCs constitute the second most frequent skin cancer, with an aggressive development in immunosuppressed subjects. It was recently demonstrated that this tumor circumvents immune effectors by down-regulating E-selectin expressed on tumor vessels. As stated in the first section, this down-regulation hinders T lymphocytes that are ready to enter in skin tumor. NO induces a lowered E-selectin expression on endothelial cells. SCC have been demonstrated having MDSC that produce NO, thus a iNOS+/CD11b+/CD33+/CD11c-/HLA-DR- phenotype. *In vitro*, MDSC isolated from SCCs produced transforming growth factor-β (TGF-β) and arginase in addition to NO. All these molecules contribute to the lowered expression of E-selectin on endothelial cells.

In this type of cancer, chemokine families and their receptors are also involved. MDSC isolated from the tumor have been shown to express CCR2, and SCC cells express the CCR2 ligand. This *in vivo* interaction likely increases the recruitment potential of the tumor cells, thus increasing the suppressor population of MDSC in the tumor. Using these molecular interactions, it has been reported that the use of an NO inhibitor, N(ω)-nitro-L-arginine (L-NNA), increased E-selectin expression. NO secretion, likely sustained by infiltrating MDSC, is one of the possible immune tumorigenesis trigger in SCCs. NO inhibition increases vascular E-selectin and recruits more effector T-lymphocytes [43].

The SCC initiation is in general acknowledged as pre-tumoral condition, actinic keratosis. The further development to SCC is known in 90% of UV induced lesions cases. This irradiation generates mutations in p53 gene (thymidine dimmers) which result in the uncontrolled proliferation of keratinocytes. Over-expression of p53 in squamous epithelium is directly correlated with sun exposure, a direct consequence being an altered presentation of wild type gene epitopes in MHC context [44]. An anti-tumor response anti-p53 should sustain the tumor rejection, but it is unclear whether an anti-p53 response would be a benefit for cancer patients. In addition, the clinical relevance of anti-p53 antibodies in SCC is still unclear because, in spite of the high p53 expression in tumors, patients diagnosed with SCC have a low prevalence of anti-p53 antibodies [45]. The tumor recognition and cytotoxicity mediated lysis seems to depend rather on the turnover rate of p53 and not on the p53 expression level. Tumors with high p53 degradation rate displayed an increased number of epitopes designated to be recognized by T cells [46]. Thus, there is a CD8+ response directed to p53, but there is still little data regarding CD4+ cellular response in SCC. It should be mentioned that T lymphocytes with CD4+ phenotype isolated from normal individuals recognize peptides derived from the central domain of protein p53. CD4+ T lymphocytes infiltrate SCC tumors and could play a role in tumor regression [47]. The tumor protects itself from anti-tumoral immune effectors by increasing FasL expression, evading thus an immune response. Cellular receptors that mediate apoptosis also take part in the immune evasion. Therefore, high expression of FasL and TRAIL in SCC prevents the effectors cells to attack, and, moreover, this expression is misleading TRAIL-R1, -R2, Fas and FLIP, and accounts for tumor resistance to apoptosis. In addition, FasL in SCC is active and induces apoptosis in Fas positive cells [48].

SCC is a skin cancer with immune background and aggressive behavior in immune suppressed subjects and needs to be further studied by immunomics domain.

Cutaneous Lymphoma

Primary cutaneous lymphomas represent a group of lymphoproliferative disorders being considered the second most important cutaneous manifestation of non-Hodgkin extraganglionary lymphomas. The most frequent (over 70%) are primary cutaneous T cell lymphomas which include mycosis fungoides (MF) and Sezary syndrome (SS), and primary cutaneous lymphoproliferative disorders with CD30+ cells.

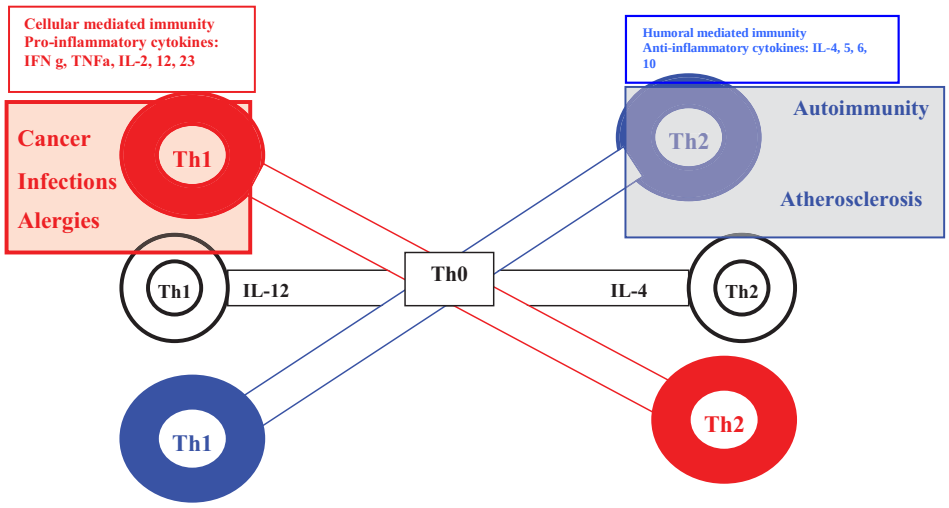

Fig. (2). Th1-Th2 physiological balance. When immunity is equilibrated Th1 and Th2 mediated processes are in equilibrium. Tumorigenesis is favored when Th1 surpasses Th2, hence pro-inflammatory cytokines prevail: IFNgamma, TNFalpha, IL-2, 12, 23. If Th2 surpasses Th1 humoral immunity controls the response and anti-inflammatory cytokines: IL-4, 5, 6, 10 can favor autoimmunity. Th1 and Th2 can be generated from null T helpers upon IL-12 and respectively IL-4 action.

Cutaneous T cells lymphomas (CTCL) are being researched, with a focus on the search and test of new skin immunobiology concepts. The behavior of malign lymphocyte is heavily influenced not only by an abnormal proliferative potential but also by local tumor environment [49]. In advanced stages of disease, especially in MF cases, tumor progression is sustained by a gradual decrease of T cell CD8+ response in parallel with a prevalence of TCD4+ response and a Th2 cytokine pattern. The T cell route and further residence in skin is sustained by cell surface expression of adhesion molecules and/or chemokine receptors such as CLA, CD62E, CCR4, CCR10, CCL17/TARC and CCL27/CTACK. The intraepidermal collection of lymphocytes is closely related to cytokines secreted by LC, which attract and lead lymphocytes to the epidermis. All abnormal changes in cytokine and lymphocyte subpopulation ratios during tumor progression could be also explained by the tumor immunoeditting theory. This hypothesis presumes three major steps in cutaneous lymphoma development: elimination, equilibrium and evasion. During elimination step, the immune surveillance prevails but a silent clone of the T cell always proliferates and conducts to monocyte activation and IFN gamma secretion. Hence, CD8+ T lymphocytes are activated and the array of Th1 cytokines is produced. At equilibrium, the anti-tumor response and malign T proliferation are equally run. The Th1 cytokine pattern is predominant and tumor infiltrate comprise both CD8+ T cells (anti-tumor effectors) and CD4+ T cells (pro-tumor cells). In addition, the equilibrium phase is specific to early stages of T cell lymphomas (clinical stages I and II). As abnormal proliferation continues, taking into account the more genetically instable background, new tumor antigens are generated (neo-antigens) and tumor cells transition to the evasion phase. Several molecules with immunosuppressive roles (IL-10, FasL or CTLA4, *i.e.*) are expressed more intensely on malignant lymphocyte and contribute thus to the immune evasion of lymphomas. As a result of immune evasion, the abnormal cell proliferation is moved to distal sites, lymph nodes and other anatomic sites, and metastasis phases occur [50]. The cytokine pattern picture in T cell lymphoma is correlated to the disease stage. Thus, in early stages of MF, skin lymphocytes secrete a Th1 cytokine pattern (IFN-gamma, IL-2, IL-12) and in late stages, the cytokine secretion is commuted to a Th2 pattern of cytokines (IL-4, IL-5, IL-10 and IL-13) (Fig. **2**). In the late stages of MF, the high level of IL-10 is correlated with a low secretion of IFN-gamma thus inhibiting an eventual anti-tumor response. In addition, the T cell subpopulations could control the SS development by secretion of a cytokine panel. As SS progresses, there is a decline in IL-2 and IFN gamma secretion along side with an increase of CTLA4 expression. In addition, the levels of IL-5 and IL-10 are significantly increased compared to normal values. Regarding increased secretion of IL-15, this finding is correlated with a reduced apoptosis of malignant lymphocytes, clonal expansion of CD4+ T cells, and eosinophils recruitment. Eosinophils recruitment contributes to prurient characteristic in SS. A subset of CD4+ T lymphocytes, named Th-17 are the main provider for IL-17, IL-21 and IL-22 secretion. These Th-17 cytokines act directly on keratinocytes, which in turn will produce IL-6 and IL-8, the main attractants for neutrophils and other T cells in the skin. Recently, IL-16 has been considered an attractant for CD4+ T lymphocytes thus promoting T cell lymphoma development. In advanced stages, both MF and SS have an overall immune dysfunction that can raise the risk of infections, actually the major cause of death in patients with CTCL. The disturbances in cytokines balance exhaust the T cell benefic populations by releasing their TCR repertoire or by a sustained inhibition mediated by CTLA4 [51].

CTCL is characterized by clonally derived malignant T cells with deregulated skin-homing receptors. As stated in the first section, DCs are one of the main immune regulators in skin. DCs are one of the immune-surveillance cells in the anti-tumoral immune response. In T-cell lymphoma, with its variants MF and SS, the distribution and maturation of DCs were recently reported. The following subsets were studied: LC with langerin/CD207+, DEC-205/CD205+ phenotype,

dermal DCs with DC-SIGN/CD209+, CD205+ phenotype and plasmacytoid DC with BDCA-2/CD303+ phenotype. Authors found that in comparison with normal skin, plasmacytoid DCs, LCs and dermal DCs, were increased, while DCs were found to be phenotypically immature. A mature phenotype of DCs may induce an efficient anti-tumor immune response, while the immature phenotype likely induces tumor tolerance [52]. In fact, the MF variant is a malignancy of resident effector memory T cells, while the SS variant is a central memory T lymphocytes malignancy [53]. In this skin cancer, malignant T cells were proven to express high level of chemokine receptor CCR4, a receptor with skin-homing capacity. CCR4 is also highly expressed on Tregs and through this receptor it can migrate into tumors and sustain the immune-suppression and further the tumor immune escape. Recently an anti-CCR4 monoclonal antibody (mAb), mAb1567 was tested in a murine experimental model. This mAb1567 recognizes CCR4 and can inhibit malignant T cells that are CCR4+. This immune-drug induced several other effects, including enhancement of complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and NK cytotoxicity. This antibody was humanized (mAb2-3) and selected for preclinical tests as a novel immunotherapy for cutaneous lymphoma, but as of this date no clinical trial results have been released [54].

Rare Skin Tumors – Merkel Cell Carcinoma

Merkel cell carcinoma (MCC) is a very rare and aggressive skin cancer of neuroendocrine origin, which, as other skin cancer pathologies, relies on immunosuppressant condition [55]. The discovery of Merkel cell polyomavirus (MCPyV) in 2008 [56] has added a new vision on MCC's pathogenesis, suggesting a strong involvement of MCPyV in MCC onset. There are few MCC reported cases when this immune suppression is surmounted by an inexplicable recovery of immune functions, leading to spontaneous regression of the tumor [57]. The latest insights in humoral and cellular immunity in MCC have offered supplementary information about host-immune interactions. In MCC patients, high titers of IgG antibodies raised to viral capsid proteins were detected [58], detection that has been related to an improved progression-free survival in MCC cases [59]. Additionally, antibodies raised to viral T oncoprotein could be considered a biomarker for disease burden. A high titer of these antibodies was detected in MCC patients who received therapy, finding that indicated disease progression before symptom's appearance [60]. Generation of T oncoprotein specific antibodies indicate an active adaptive immunity by recognition of viral proteins exposed to tumor cells.

The content of cellular immune infiltrate in MCC directly influences the patient's survival, thus a high percentage of intratumoral T CD8+ lymphocytes indicates a better prognosis and survival [61]. Otherwise, T lymphocytes dysfunction favors the tumor immune escape and, in elder population, these immune dysfunctions are augmented by aging. Besides host condition, UV radiation affects skin immune system by attracting Treg cells to tumor site, diminishing lymphocyte mediated cytotoxicity and functionally disturbing antigen presenting machinery of APC cells [62]. Taking into account the viral role in MCC pathogenesis, as a result of chronic viral antigen exposure, T CD8+cells could display an overwhelmed phenotype associated with reduced effectors function leading finally to a reduction or even to a clonally deletion of T CD8+cells [63].

The immune reaction in MCC is sustained by effector T cells, central memory, and by Tregs that infiltrate the tumor. Unfortunately, the infiltrating T lymphocytes have a reduced expression of CD69 and CD25 and hence low activation. The involvement of immune elements was proven when *in vitro* MCC tumors were subjected to IL-2 and IL-15. This *in vitro* treatment induced lymphocyte T activation, proliferation, and increased cytokine production, leading to tumor cells apoptosis. Isolating TILs, authors have demonstrated that these lymphocytes showed a specific TCR repertoire and an increased expression of one member of the tumor necrosis factor receptor family, TNFRSF9, known as CD137. *In vivo* experiments have shown that, when implanted to immunodeficient mice, these tumors grew only when tumor-specific T lymphocytes were killed prior to implantation with denileukin diftitox. This *in vivo* experiment proved the control developed by intra-tumoral T lymphocytes $CD4^+$ and $CD8^+$ $FOXP3^+$ [64]. CD28 family of receptors comprises PD-1 (Programmed death-1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), inducible costimulator (ICOS), and Band T-lymphocyte attenuator. All these receptors are important in immune tumor control. CD28 is a costimulatory receptor which enhances T activation, while CTLA-4 decreases T activation. Another family member, PD-1, has an inhibitory action on both T and B lymphocytes, being involved in peripheral tolerance. PD-L1 and PD-L2 are ligands for PD-1, expressed on a range of cells, among which skin is one of the main organs where this couple is involved in tumorigenesis [65]. In MCC, half of the resting T cells express PD-1, while its ligands PD-L1 and PD-L2 were reported as expressed by DCs and macrophages associated to MCC.

All in one, in MCC, the immune control of tumorigenesis is hindered by the existence of T suppressed action; therefore drugs that can induce immune stimulation, block T suppressors and/or inhibit PD-1 mediated signaling are to be considered future immune-drugs in MCC [64].

Melanoma

Cutaneous melanoma and its immune-related tumorigenesis mechanisms and biomarker discovery efforts have been the subject of the highest number of publications focusing on skin cancers. Cutaneous melanoma is of high interest to both clinicians and researchers. It is the most deadly skin cancer, with death tolls and societal burden rising each year. Cutaneous melanoma is a cancer that still has no validated immune markers although it has the most studied immune response out of all skin cancers.

In melanoma, the immune milieu consists mainly of Tregs, natural killer T cells (NKT), and distinct subsets of immature and mature DCs. All these cellular components comprise the immunosuppressive network in cutaneous melanoma [66]. The stated failure of immune-therapy in this type of cancer can be explained by the fact that tumors can overcome immunosurveillance following 'immunosculpting' by the immune system. This notion, immunosculpting, was

introduced several years ago, and explains the tumor-induced immune tolerance and suppression, the loss of immunogenicity observed in advanced-stages of melanoma. This effect can be the result of the immune distortion and immune-induced alterations that can contribute to cancer pathogenesis [67]. When we analyze melanoma tumorigenesis, it is accepted that melanocytic proliferation is restrained by the immune system; the mechanisms that underlie immune suppression, and allow the development of cutaneous melanoma, are still under intense study [68]. As ascertained in the first section, tumor microenvironment nurtures several pro-tumor mechanisms: reduced chemokine-mediated trafficking of effector cells, negative regulatory pathways that inhibit T-cell function, the already known immune escape of cancer cells and their adaptation to a vigorous immune pressure [69]. The failure of antitumor immune responses resides in the local immunosuppressive cells and factors. In this context, immature DCs, neutrophils, Tregs, myeloid-derived suppressor cells and tumor-associated macrophages have important roles [70].

A hormone (alpha-melanocyte-stimulating hormone) involved in skin pigmentation is connected to inflammation and immunomodulation. On human CD8+ T lymphocytes, this hormone has a specific receptor (melanocortin-1 receptor) that is critically involved in the MHC class I-restricted cytotoxic function of CD8+ T cells [71].

In this complex cellular microenvironment, interactions between melanocytes and all these immune-generated factors and immune cells can lead to the promotion of malignant transformation and to acquiring the invasive potential of a skin tumor.

The purposeful age of melanoma immunobiology has started with two major findings, first the autologous melanoma cells immune recognition by T cells in HLA-I immune context, and second, the discovery of specific antibodies for defined molecules expressed by melanoma cell. The progresses registered in T cell phenotypic portrayal account for the development of melanoma immunobiology, being supported as well by elucidation of the DC role in tumor antigens processing. The T cell response in melanoma has some characteristics, thus, both T cells from peripheral blood and lymph nodes recognize melanoma cells in HLA-I context. Besides this, both T CD4+ and CD8+ circulatory cells recognize almost all classes of antigens: antigens with different tissue distribution, antigens shared by melanoma and normal cells or antigens restricted to specific tumors. These T cell can recognize the heterogeneity of antigens from the same tumor developing in an individual patient. Once elicited, the cellular mediated immune response in melanoma is continued and maintained by chemo-attractants such as Stat3, MCP-1, GRO-a, IL-8, MIG, IP-10 or RANTES generated by tumor infiltrating immune cells [72]. For example, Stat3 is a transcription factor constitutively activated in several human cancers affecting the recruitment of other immune cells. In an experimental murine model of cutaneous melanoma, the natural activity of Stat3 is associated with tumor growth and reduction of T cell tumor cellular infiltration. Blocking of the Stat3 signaling pathway in melanoma cell can lead to an increased expression of multiple chemo-attractants, the main effect of which is the increase of migration of lymphocytes, NK cells, neutrophils and macrophages to tumor sites. Therefore, Stat3 blocking induces tumor cells to produce a plethora of soluble factors able to activate macrophages to produce toxic species such as NO. TAM is a cell population with a dual nature in tumoral environment. There is a balance between immune signals generated by TAM, these signals are dependent on "who is listening"- namely cells that will either stimulate or decrease the tumor growth. The tumor reducing growth could be achieved by several mechanisms including direct cellular cytotoxicity, antibody-mediated cellular cytotoxicity, or secretion of macrophage chemotactic factors and colony stimulation factors such as GM-CSF. Pro-tumoral effectors decrease macrophage recruitment at the tumor sites, increase melanoma cell adhesion to the extracellular matrix, increase in COX2 expression and all these mechanisms being critical for melanoma development [73].

In melanoma there is a chain of immune-mediated actions that converge toward immune-suppression (Fig. **3**) [74]. In this network, innate and adaptive immune elements converge. TAM secrete indoleamine 2,3-dioxygenase (IDO), which induces an inhibition of T-cell proliferation due to tryptophan depletion. Moreover, IDO recruits regulatory T cells (FOXP3+) into the developing tumor. Tregs secrete TGF-beta and recruit more Tregs, the suppression induced on the effector couple CD4–CD8 increases, and therefore the immune control of tumor development decreases. Tumoral cells by themselves secrete TGF-beta, IL-10, VEGF, PGE2 that induce DCs to secrete more TGF-beta, contributing to the conversion of CD4T cells to immune-suppressive Tregs. Skin-homing T cells have a chemokine receptor 4 (CCR4) that binds to the CCL22 (macrophage-derived chemokine) expressed by TAM, and their recruitment increases once more. Overall, a favorable microenvironment is created by these concerted actions, with the result being the proliferation of Tregs that hinder the cooperation CD4–CD8 and therefore abolishing the effector activity of antitumoral cytotoxic cells.

An overview of the intratumoral immune pattern and the known circulatory immune markers correlated with the clinical significance in the outcome of a particular skin cancer are presented in (Tables **1** and **2**).

IMMUNOMICS OF SKIN CANCER

SIS suffers a maturation process along with the skin development; this development comprises epidermal proliferation, maturation, and continuous adjusting of the epidermis and dermis compartments. SIS maturation represents the immune tolerance for self antigens while developing a robust and efficient immune response to non-self. Using an experimental mouse model and proteomics technologies SIS elements were identified. Two-dimensional electrophoresis (2DE) and sequencing by peptide mass fingerprinting with matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-ToF-MS) identified 25 proteins, out of which 3 were keratinocyte differentiation and proliferation markers, cyclophilin A, epidermal fatty acid binding protein 5 and stefin A. Two isoforms of stefin A, a member of the cystatin family, were identified in neonatal skin,

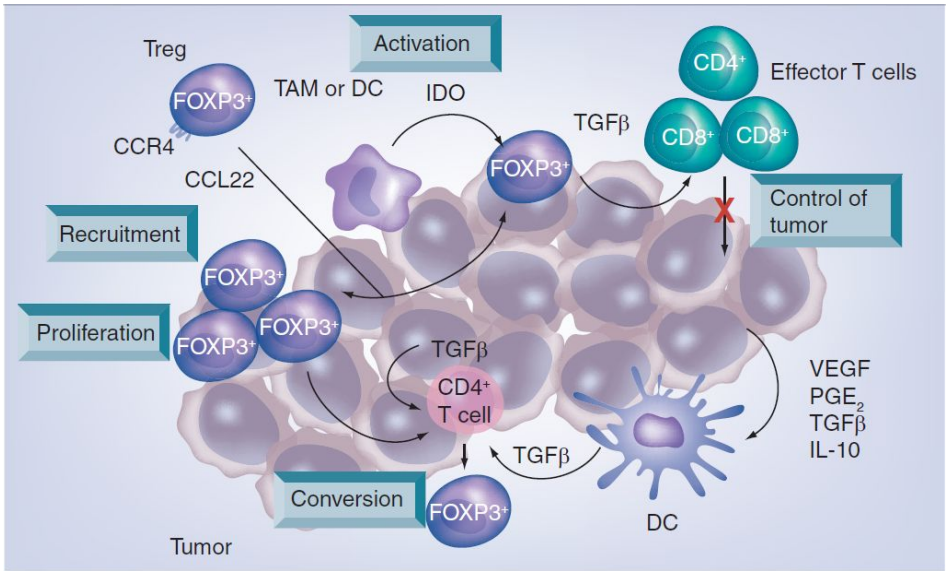

Fig. (3). Immune suppression developed in melanoma tumor (copyright permission for reproduction from [74].
Macrophages secrete indoleamine 2,3-dioxygenase (IDO) that induce an inhibition of T cell proliferation due to tryptophan depletion (activation). Moreover IDO recruits regulatory T cells (FOXP3+) at the tumoral site. Recruiting more TGF beta-secreting Tregs the suppression induced on the effector couple CD4-CD8 increases and therefore the control of tumor development decreases. Tumoral cells by themselves secrete TGFbeta, IL-10, VEGF, PGE2 that induce DCs to secrete more TGFbeta contributing to the conversion of CD4+ T cells to Tregs phenotype enhancing the cellular immune suppression (conversion). Skin-homing T cells CC-chemokine receptor 4 (CCR4) binds to the CCL22 (macrophage-derived chemokine) of the tumor associated macrophages (TAM) and are recruited to the tumoral site (recruitment). On the whole, a favorable microenvironment is created by the concerted action that has as a result the proliferation of Tregs that hinder the cooperation CD4+-CD8+ and therefore abolishes the effector activity of anti-tumoral cytotoxic cells.

Table 1. Tumour tissue immune pattern and their clinical significance in skin cancer.

Skin Cancer	Immune Cells/Molecules	Comments	References
BCC	TIL mainly T and LC	Correlation with tumor size	[36]
	TAM in tumor infiltrate	Increased invasiveness	[40]
SCC	Intratumoral suppressor population of MDSC	Tumor aggressiveness	[43]
	TIL with CD4+ T	Tumor regression	[47]
CL	Decrease in CD8+ T response, increase in TCD4+ response and a Th2 cytokine pattern	Tumor progression	[50]
	Th1 cytokine pattern (IFN-gamma, IL-2, IL-12)	Early stages of MF	[51]
	Th2 pattern of cytokines (IL-4, IL-5, IL-10, IL-13)	Late stages of MF	[51]
MCC	High percentage of intratumoral T CD8+	Better prognosis and survival	[61]
Melanoma	Immature DCs, neutrophils, Tregs, myeloid-derived suppressor cells and TAM	Bad prognosis	[70]

proposed to be involved in skin and SIS development [75]. Recently, stefin A was reported as involved in neoplastic transformation of the epithelium. Moreover, an imbalance in cathepsins - cystatins can be involved in hindering immune cell effector functions and facilitating tumor cell invasion [76].

In cutaneous lymphoma, 1- and 2-dimensional Western blot and proteomics-based protein identification tests were used to assess patient sera. Patients' sera were found positive to some tumor antigens, further identified from silver-stained gels by MALDI-ToF-MS. The authors found 14 antigens, out of which only vimentin had been previously reported in cutaneous lymphomas [77].

Using a proteomic approach for serum proteomic maps in cutaneous melanoma, it was recently proven that levels of transthyretin (TTR) and angiotensinogen (AGT) increase, while vitamin D binding protein (DBP) was decreased. These proteins returned to normal values after surgical

Table 2. Circulatory immune markers in patients diagnosed with skin cancer.

Skin Cancer	Immune Cells/Molecules	Comments	References
BCC	Low number of circulating T lymphocytes	Correlation with tumor size	[33]
CL	Th1 cytokine pattern (IFN-gamma, IL-2, IL-12)	Early stages of MF	[51]
	Th2 pattern of cytokines (IL-4, IL-5, IL-10, IL-13)	Late stages of MF	
MC	High titers of anti-viral IgG antibodies	Improved progression-free survival	[58, 60]
Melanoma	Low CD4+/CD8+ ratio	Marker correlated with stage	[74]
	High serum IL-6, IL-10 combined with LDH	Marker for advanced stage disease	[74]

removal of stages I and II cutaneous melanoma tumors. DBP protein is highly involved in immune response. Due to alpha-N-acetylgalactosaminidase secreted by melanoma cells, DBP is deglycosylated, and the immune pathway in which DBP is involved is deregulated and immunosuppression is sustained [78]. It was recently reported that in human T cells, DBP modulates T cell responses, and this action is performed by hindering the uptake of inactive 25-hydroxyvitamin D3 by DCs. It is one of the first publications demonstrating that the level of free 25-hydroxyvitamin D3 available to DCs induces an immune balance and thus T mediated inflammatory/regulatory immune responses [79].

In cutaneous melanoma, serum alpha-N-acetylgalactosaminidase was found significantly increased in stage III malignancies, while found normal in early stages. This is another explanation of induced immune-suppression in advanced stage [78].

Proteomics research has identified molecules exclusively expressed in neonatal skin, including stefin A, and peroxiredoxins (see above). Vitamin D has a modulating role for immune response in the skin, a process that likely appeared in early embryogenesis. In transgenic mice experimental models, it has been reported that following a single high dose of UV radiation, hepatocyte growth factor/scatter factor or TPras were identified, these factors can lead to immune suppression in skin and in adult life to melanoma. UV-induced immunosuppression has a pivotal role in melanoma development [80].

Immunomics in Skin Cancer Therapy

Melanoma

This skin cancer is a demonstration of a battle between immune responses and the developing tumor cells, and thus immunotherapy has had role for more than 20 years in this type of skin malignancy. Historically important, immunotherapy with IL-2 and IFNα2b has evolved into antibodies that block CTLA-4 and hence block the immune-suppression [81].

High-dose IFN-alpha is the first-line adjuvant therapy in melanoma of advanced stages. The combination of IFN-alpha or ipilimumab with tyrosine kinase inhibitors can be applied to patients who have been tested for specific mutations. Thus, ECOG 1609 clinical trials compared these immune-therapies for the treatment of early metastatic melanoma. Early metastatic melanoma patients, diagnosed in stage III-N, are likely the best set of patients for immune adjuvant therapy [82].

As it is an immune-dependent skin cancer, targeted immune-therapies are the Holy Grail for clinicians in melanoma and there are several clinical trials on immune-therapies and immune approved drugs underway. Adjuvant BRAF and/or immune checkpoint inhibition have been shown to increase survival of early stage melanoma. Predictive immune biomarkers are essential for stratifying high-risk stage III patients [83].

In a recent melanoma mouse model, total ablation of CD4 was more successful than deleting Tregs in the generation of anti-tumoral memory CD8 T cells. This new experimental data has shed new *immune-light* on how to induce effective antitumor immune responses [84].

The first immune-therapy recently approved in skin melanoma, ipilimumab (Yervoy) blocks CTLA-4 and lowers immune-suppression. Using a clear immune agent, the immune response is guided to efficiently stop suppression. The CTLA-4 over-expression detected in melanoma patients decreases the signaling network derived by antigen recognition of T cells. To overcome ipilimumab toxicity/auto-immune adverse reactions, additional immune modifiers, such as PD-1 molecule, can join the immunotherapy melanoma drugs [85,86].

In a mouse melanoma model, immune cells were studied in recurrent tumors evolution. In TILs, $FOXP3^+$ $CD4^+$ T began to express $PD\text{-}1^+$ and accounted for more than half of T $CD4^+$ cells. Effector T cells displayed high expression of PD-1, TIM-3, 2B4, TIGIT, and LAG-3 inhibitory molecules. Using an experimental therapy, combining PD-L1 blockade and Tregs depletion, regression was induced. When combined antibodies anti–PD-L1 and anti–LAG-3 were used, Treg depletion could be circumvented and clinical results were good. Primary experimental melanoma needed only Treg depletion or antibody therapy. These results underline the clear differences in immune-treating primary or relapsed skin melanomas and the use of more "intelligent" immune-therapy in relation to the tumors characteristics [87].

Immune therapy in skin melanoma has expanded lately, therefore the need to identify predictive biomarkers to personalize treatments strategies to an individual tumor or

immune system has become mandatory. Such strategies have the potential of maximizing antitumor effect while minimizing toxicity and improving clinical benefit [88, 89].

BCC

A major characteristic of the development of BCC is the immune imbalance of innate and adaptive immunity [90]. This type of cancer has two immunomodulating IFN-based agents. Exogenous IFN-alpha2b and an endogenous IFN inductor were tested in BCC patients. Immune parameters were monitored before and three months after therapy, without any BCC relapse. This is one of the few recent studies that uses immunotherapy in BCC patients, confirming the efficacy and opening an *immune-door* to BCC patients with high relapse risk [91].

Proteomics Technologies for Immunomics Biomarker Discovery

For identifying immune-related markers, diverse proteomic technologies have been used. The development of immunomics is sustained especially by microarray techniques. Antibody microarrays are one of the most popular formats of protein microarrays and maybe the first choice in immunomic discoveries; however, some issues must be resolved including the generation of specific antibody sets for each antigen target and the development of a large scale format. Peptide microarrays are the second type of array techniques to be used for immunome studies. These are suitable for functional immunomics, as antigen peptides are used as fixed probes to the support [92]. Ideally, these should be used in parallel with peptide-MHC microarrays for the high potential in identifying new clinical (bio)markers. Peptide-MHC microarray chips could be used alone for mapping the MHC-restricted T cell epitopes or in combination with peptide-based B cell epitope microarrays to study the overall adaptive immune response of a specific disease, for example [93].

Mass spectrometry is another proteomic technology that is of value in immunomics. A mouse model for skin cancer was used for label-free quantitative glycoproteomics. Mice with a mutation in the p19(ARF) gene (a type that is sensitive to skin carcinogenesis) and control mice were used for detecting differences in their plasma proteome that can indicate tumorigenesis. Using liquid chromatography mass spectrometry (LC-MS) and a developed computational framework, Corra that performs peak picking and alignment, peptides were identified relevant for the two mice groups. Then, the relevant peptides were identified by tandem mass spectrometry. Most of the identified proteins were involved in immune response activation. Besides these proteins, the ones appending to the cell cycle and apoptosis were second most abundant proteins. The authors show that a LC-MS-based workflow is an appropriate proteomic tool for immunomics discovery [94].

Dermal endothelial cells (EC) isolated from healthy donors were compared by 2D-DIGE and MS. The authors report over 150 proteins that are over expressed in human umbilical vein endothelial cells (HUVEC) in comparison to dermal EC. Fatty acid binding proteins 4 and 5 were proteins found to be different in these samples. Ingenuity® analysis showed that proteins differentially expressed in dermal EC samples interact with retinoic acid. Proteome profiles in endothelial cells from different sources, human umbilical vein, dermal or microvascular pulmonary, are different in order to sustain their specific tissue properties and immune functions [95].

For studying the skin's response to UV irradiation, recent proteomic studies used cell lines and reconstructed skin. In this study, proteomic technology has shown that UV-mediated cell injury induces oxidative response that increases the heat shock proteins. UV irradiation altered cytokeratin and cytoskeletal proteins. Upon irradiation and further keratinocytes recovery, proteomic studies revealed that tripartite motif-containing 29 (TRIM29) is increasing. TRIM29 has a transcriptional regulatory function, and has recently been shown to be involved in carcinogenesis and/or differentiation [96].

Another 2D-DIGE proteomic study showed that exposures of human keratinocytes to UVB in a repeated manner generated interesting proteome profiles. In this study, around 70 abundant proteins were identified by MS, mainly proteins involved in keratinocyte differentiation and survival. This study also identified TRIM29, later validated by Western blot and analyzed by RT-PCR. The authors conclude that TRIM29 increment, dependent on the PKCdelta signaling pathway, induces a state of protective process in a keratinocytes population instead of massive apoptosis after repeated UVB irradiation [97].

When extrapolating to "real life" UVB exposure, these processes are associated with inflammation, followed by epidermis regeneration and a modulation of the immune response sustained locally by SIS. If the irradiation is repeated, skin aging processes appear and can favor skin cancers (melanoma, squamous cell carcinoma and basal cell carcinoma) [96].

Proteomic studies have shown in BCC that connexin-43 can be involved in the low invasiveness of BCC. Increased topoisomerase IIα and reduced p21waf1 and p27kip1 in BCC were found differently expressed in comparison to SCC. In BCC and SCC, caspase-8 and -9 were equally upregulated in comparison to normal keratinocytes. In the hierarchical clustering SCC, BCC cases and actinic keratosis were grouped together [32].

CONCLUSIVE REMARKS

Tumor immunosurveillance research has offered in the last decade three major areas of study: tumor infiltrating immune cell populations, emphasizing local immune-suppression, generation of systemic anti-tumoral responses to tumor antigens highlightening immune-biomarkers in circulation (molecules and cells), and systemic immune-suppression that represent a high-risk group of patients for developing cancer [67]. For example, in immune suppressed patients there is an increased risk of developing skin and lip cancer, lymphomas, and Kaposi's sarcoma but not the most common malignancies such as breast and prostate cancers [98]. One reasonable explanation is that skin has multiple layers of immune defense mechanisms and moreover skin represents the equilibrium organ between the external environment (including carcinogens) and internal homeo-

stasis. Being the largest immune organ, skin *covers* the largest aggression surface and must cope with the most complex array of immune mechanisms.

The cutaneous immune reactions are performed in the framework of SIS where keratinocytes and dermis provide a structural context in which such immune responses are initiated and where pathological lesions may develop. Embryonic development of the skin takes advantages of the directional guidance of cells *via* gradients of chemokines. This guided process becomes crucial in skin cancer dissemination and in generation of efficient anti-tumoral immune responses. The *Human Genome Project* that triggered the *Human Proteome Project* pushed the development of proteomics technology [99]. High throughput and screening technologies identified biomarkers that were put in use for disease prognosis and immune therapies. Scrutinizing the immune system and the immune responses developed in skin cancer, it is obvious that immunomics has the potential to deliver new, individually tailored therapeutical approaches [82]. Immunomics has been developing as a necessity to comprehend the continuously gathering of immunological data in relation to human pathology. Immunomics is a domain that is not blocked in one field; it overlaps basic immunology, the appended genomics and proteomics and makes thorough use of computational biology. In this manner it addresses pathophysiology and gives the medical world important clues regarding immune diagnostics and monitoring, developing new immune-therapies. Immunomics has reaching probably its ten's birthday but is accumulating complex and diverse data [100], hence the generation of a new rising field *Immunoinformatics* [101].

Due to the complexity of interactions between skin tumors and the immune system, suppressing antitumor responses and the discovery of predictive biomarkers has been particularly challenging [89]. The specific role of immune cell populations in melanoma and in non-melanoma skin cancers continues to be explored *in search of new biomarkers and new immune networks*.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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ABBREVIATIONS

2DE	=	Two-dimensional electrophoresis
ADCC	=	Antibody-dependent cellular cytotoxicity
Ag	=	Antigen
AGT	=	Angiotensinogen
APC	=	Antigen-presenting cells
BCC	=	Basal cell carcinoma
CC (beta)	=	Beta-chemokine
CCL(2-28)	=	Chemokine receptor ligand
CCR (2,4,5,6,10)	=	Chemokine-beta receptor
CD44v3	=	CD44 splice variant 3
CDC	=	Complement-dependent cytotoxicity
CL	=	Cutaneous lymphomas
CLA	=	Cutaneous lymphocyte antigen
COX2	=	Cyclooxygenase 2
CTACK	=	Cutaneous T-cell-attracting chemokine
CTL	=	Cytotoxic T lymphocytes
CTLA4	=	Cytotoxic T Lymphocyte-Associated Antigen 4
CXC (alpha)	=	Alpha-chemokine
CXCR	=	Chemokine-alpha receptor
CXCL10	=	CXC chemokine ligand 10
DBP	=	Vitamin D-binding protein
DC	=	Dendritic cell
EGFR	=	Epidermal growth factor receptor
Fas	=	TNF receptor superfamily, member 6
FLIP	=	Fas-ligand ICE inhibitory protein
FOXP3	=	Forkhead box protein P3
G-CSF	=	Granulocyte colony-stimulating factor
GM-CSF	=	Granulocyte-macrophage colony-stimulating factor
gp100	=	Glycoprotein 100
ICAM-1	=	Intercellular adhesion molecule-1
ICOS	=	Inducible costimulator
IDO	=	Indoleamine 2,3-dioxygenase
IL	=	Interleukin
iNOS	=	Inducible NO synthase
IP-10	=	Interferon-inducible protein-10
HUVEC	=	Human umbilical vein endothelial cells
LAG-3	=	Lymphocyte activation gene- 3
LC	=	Langerhans cells
LC-MS	=	Liquid chromatography mass spectrometry
MALDI-ToF-MS	=	Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry
MCC	=	Merkel cell carcinoma
MCPyV	=	Merkel cell polyomavirus

M-CSF	=	Monocyte-macrophage colony-stimulating factor
MCP-1	=	Monocyte Chemotactic Protein-1
MIG	=	Macrophage induced gene
MHC	=	Major histocompatibility complex
MF	=	Mycosis fungoides
MO-MDSC	=	Myeloid-derived suppressor cells
NKT	=	Natural killer T cells
NO	=	Nitric oxide
PBMC	=	Peripheral blood mononuclear cell
PRDX	=	Peroxiredoxin family
PECAM-1	=	Platelet Endothelial Cell Adhesion Molecule 1
PD-1	=	Programmed cell death 1
PD-L1/2	=	Programmed cell death ligand 1/2
RANTES	=	Regulated on Activation Normal T Cell Expressed and Secreted
RORγt	=	Retinoic acid-related Orphan Receptor γT
ROS	=	Reactive oxygen species
SALT	=	Skin-associated lymphoid tissue
SCC	=	Squamous cell carcinoma
SIS	=	Skin immune system
SLN	=	Sentinel lymph node
SS	=	Sezary syndrome
STAT-1	=	Signal transducer and activator of transcription-1
TAM	=	Tumor-associated macrophages
TARC	=	Thymus and activation-regulated chemokine
TCR	=	T-cell receptor
TGF	=	Transforming growth factor
Th	=	T-helper
TIA-1	=	T-cell intercellular antigen-1
TIL	=	Tumor-infiltrating lymphocytes
TIM-3	=	T cell immunoglobulin and mucin domain
TLR	=	Toll-like receptor
TNF	=	Tumor necrosis factor
TNFR	=	Tumor necrosis factor receptor family
TNFRSF9	=	Member of the TNFR family
TRAIL-R1/2	=	Tumor Necrosis Factor-related Apoptosis-inducing Ligand 1/2
Treg	=	Regulatory T cell
TRIM29	=	Tripartite motif-containing 29
TSLP	=	Thymic stromal lymphopoietin
TTR	=	Transthyretin
VCAM	=	Vascular cell adhesion molecule

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