Resposta Imunológica no Melanoma: Base para a Compreensão do Papel da Imunoterapia com Inibidores de "Checkpoints" Imunológicos

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RESUMO – O conhecimento do processo de evolução tumoral é essencial para compreender os alvos terapêuticos no controle da doença. A forma como o sistema imune influência o desenvolvimento e a progressão do cancro é uma questão desafiante na área da imunologia. Atualmente reconhece-se o papel paradoxal do sistema imunológico neste processo: por um lado protege contra o crescimento tumoral, destruindo células exprimindo antigénios tumorais "aberrantes", por outro pode favorecer a sua progressão, selecionando células tumorais que escapam à vigilância imunológica e são capazes de sobreviver num hospedeiro imunocompetente. Esta observação deu origem ao conceito de *"cancer immunoediting"*, que explica a influência do sistema imune na progressão tumoral. Tendo em conta algumas observações associadas ao melanoma, como por exemplo, o desenvolvimento de vitiligo, a possibilidade de regressão e a correlação com a imunossupressão, este tem sido considerado um exemplo de tumor imunogénico, cujo mecanismo patofisiológico reconhecido até à data se enquadra no conceito de *"immunoediting"*. Reconhecida a importância de CTLA-4 (antigénio linfócitário T citotóxico) e PD-1 (proteína de morte celular programada) como *"checkpoints"* imunológicos na regulação da atividade das células T em resposta à progressão tumoral, estas moléculas têm sido considerados alvos terapêuticos importantes no tratamento do melanoma avançado. O presente artigo pretende rever sucintamente o processo de evolução tumoral e respetiva interação com o sistema imune, bem como o mecanismo de ação dos *"checkpoints"* inibitórios por forma a melhor compreender os novos alvos da imunoterapia no melanoma avançado, que serão revistos em trabalho futuro.
PALAVRAS-CHAVE – Imunoterapia; Melanoma/imunologia; Melanoma/tratamento; Receptor de Morte Celular Programada 1;Vigilância Imunológica.

Immune Response in Melanoma: A Basis to Understand the Role of Immunotherapy with Immune Checkpoint Inhibitors

ABSTRACT – *The knowledge of the pathophysiology of tumour progression is crucial to understand the therapeutic targets in order to control the disease. The mechanisms used by the immune system to affect cancer development and progression has been a challenging question in immunology. It is now postulated that immunology plays a dual role in this process: it protects against tumour growth, destroying "aberrant" tumour cells, but may also promote tumour progression by selecting tumour cells that are able to escape the immune response and survive in an immunocompetent host. These findings gave rise to the concept of "cancer immunoediting", which explains the influence of the immune system on tumour progression. Several observations like immunosuppression as a risk factor for melanoma, the possibility of partial or complete regression of primary tumour and development of vitiligo, have suggested that melanoma is an immunogenic tumour but a successful tumour evolution can occur in the light of*

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the "immunoediting" concept. Immune checkpoints, cytotoxic T lymphocyte antigen (CTLA)-4 and programmed cell death (PD-1), were recognized to have important roles in regulating T cell responses during tumour development and were proven to be effective targets in treating advanced melanoma. This article will briefly review the process of tumour evolution and its interaction with the immune system as well as the mechanism of action of the immune checkpoint inhibitors to understand better the new targeted immunotherapies for advanced melanoma, that will be further discussed.
***KEYWORDS** – Immunologic Surveillance; Immunotherapy; Melanoma/immunology; Melanoma/therapy; Programmed Cell Death 1 Receptor.*

INTRODUCTION

The way the immune system influences tumour development has long been a subject of discussion. In the 50's, Burnet highlighted the importance of adaptive immunity in preventing cancer development in immunocompetent hosts, originating the concept of "cancer immunosurveillance".[1] Notwithstanding, further experimental studies showing that cancer susceptibility in immunocompetent mice was similar to immunodeficient mice[2,3] pulled down the "cancer immunosurveillance" hypothesis. The idea of immunity and tumour progression had started with the demonstration of tumour antigens.[4] Later, other authors argued that tumours result from transformation of normal cells with unregulated growth control, thereby tumour immunity would resemble immunity to normal tissues with the immune system recognizing tumour antigens as "self".[5] Moreover, persistent activation of the innate immune system in pro-inflammatory states may promote cancer development in this tumour microenvironment.[6,7] Nevertheless, the concept of cancer immunosurveillance was rekindled by the discovery of the effect of interferon-gamma (IFN-γ) in promoting immunologically induced rejection of transplanted tumour cells, showing the crucial role of IFN-γ in promoting tumour cell recognition and elimination.[8] Additional studies documented the importance of immune system in cancer suppression.[9]

Based on the previous findings, it is currently warrantable that immune system has a dual role in cancer development. As it protects against foreign pathogens and controls inflammation it eliminates certain tumour cells and, consequently, the link between immunosuppression and increased cancer risk is widely established.[10] On the other hand and despite a normal host immune system, tumours develop evasion mechanisms in which the immune system shapes the immunogenic phenotype of the tumor preventing their antigens to be immune targets,[11] therefore avoiding immunological elimination of the tumour cells.[12] Cancer immunoevasion, through the modification of tumor cells or its microenvironment by the immune systems is considered within the process of immunoediting and has been regarded as an emergent "hallmark of cancer".[13] Thereby the term "cancer immunoediting" has been used to describe the dual role of immune system on cancer progression: host protection ("cancer immunosurveillance") *versus* tumor promotion ("immunoevasion").[11,12]

Due to new insights on the immune escape mechanisms, immunotherapy has recently emerged as an effective treatment approach.[14] Particularly in melanoma that very likely progresses through the stages of cancer immunoediting,[15] the recognition of CTLA-4 and PD1/PD-L1 as targets to immune checkpoint inhibitors revolutionized the treatment of this aggressive tumour.[16-18] Herein we briefly review the process of cancer immunoediting and the mechanisms of action of immune checkpoints inhibitors to contextualize immunotherapy in advanced melanoma.

1. CANCER IMMUNOEDITING

Cancer immunoediting comprises 3 different phases: the elimination phase, the equilibrium phase and the immunological escape phase[19,20] (Fig. 1).

Elimination: The elimination phase underlies the concept of "cancer immunosurveillance", in which both innate and adaptive immune response work together to eliminate detected foreign tumour cells. Main pathways crucial to recognize and eliminate the most immunologically vulnerable antigenic tumour cells include recognition molecules such as NKG2D important for NK cells, host effector molecules like interferon type I (IFN-α/β), interferon type II (INF-γ), perforin and Fas/FasL, and effector cells like CD4+ and CD8+ T cells and B cells.[9,19] In addition, NK cells and macrophages from innate immune system improve surveillance of senescent tumour cells.[9] Of outmost importance in this phase is the controlled release of pro-inflammatory and immunomodulatory mediators, which paradoxically may establish a microenvironment that facilitates tumour growth.[21] Thus, a coordinated and balanced work of innate and adaptive immunity is needed to protect the host against a developing tumour and reach the primary endpoint of the elimination phase, a complete destruction of tumour cells.

Equilibrium: The equilibrium phase is poorly understood since it is very difficult to replicate in animal models.[19] Nevertheless, it is postulated that the adaptive immune system prevents tumour cell outgrowth and at same time sculpts the immunogenicity of the tumour cells.[22] Perhaps the equilibrium is the longest phase, were the immune system keeps residual tumour cells in a functional state of dormancy.[20] Using a mouse model, Koebel *et al*[22] demonstrated that tumour cells in equilibrium are highly immunogenic (unedited tumour cells), whereas those spontaneously exiting equilibrium and that become growing tumours have attenuated immunogenicity (edited tumour cells). They also showed that the equilibrium phase is maintained solely by the adaptive immunity, while the eliminating phase requires the concerted action of adaptive and innate immunity.

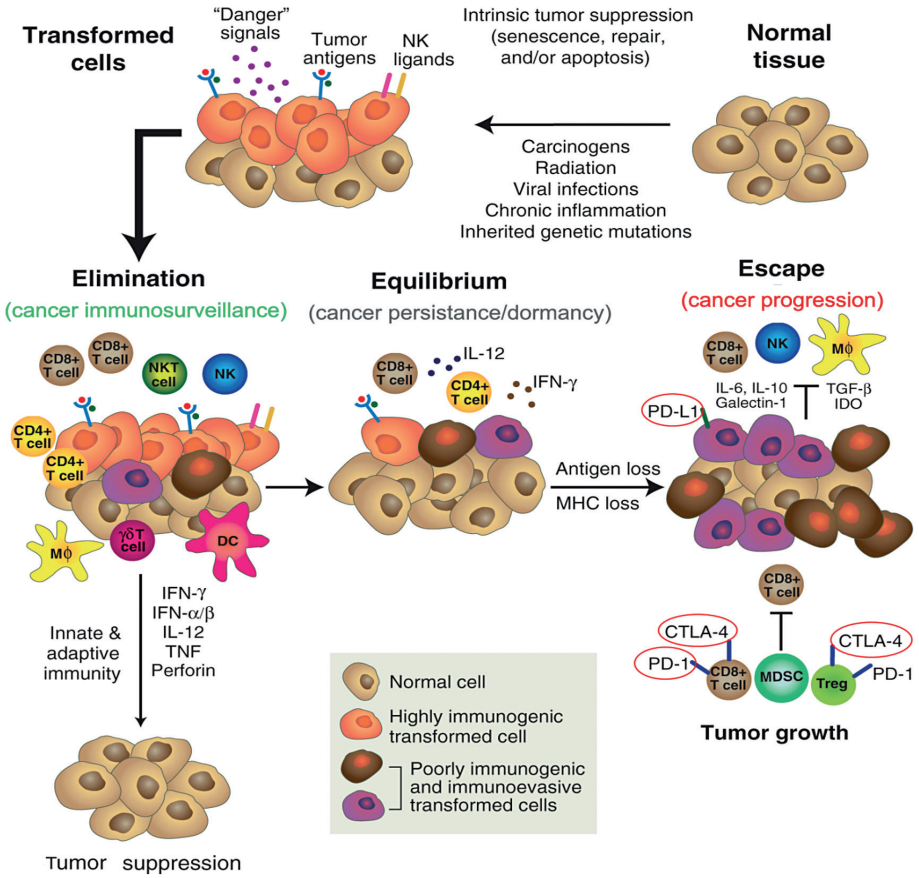

Figure 1 - The cancer immunoediting concept and the respective three sequential phases: elimination, equilibrium, and escape. The interaction between extrinsic factors, the innate/ adaptive immunity of the host and the factors derived from the tumor, promote the tumour growth (Figure adapted from Vesely MD *et al*[9] and Schreiber RD *et al*[20]).

Escape: In the escape phase tumour cells that have acquired the ability to evade immune recognition progressively grow as visible tumours. Several mechanisms both from tumour cells and the microenvironment have been proposed to explain immunological escape.[9,11,14,23]

Immune recognition of tumour cells can be reduced by loss of tumour antigens perhaps in association with genetic instability,[23] survival and emergence of tumour cells lacking "strong" antigens and by the loss of major histocompatibility complex (MHC) class I expression, preventing T cell recognition of tumour antigens. Resistance to immune cytotoxic effects can increase due to the activation of pro-oncogenic transcription factors (e.g: MITF – melanogenesis associated transcription factor, STAT3 – signal transducer and activator of transcription 3, RUNX – runt related transcription factor, NF-kB – factor nuclear kB) or to the expression of anti-apoptotic molecules (e.g: BCL-X -regulator of programmed cell death, FLIP - Fas-associated death domain-like interleukin-1-converting enzyme-like inhibitory protein) or lack or aberrant expression of MHC class I or II, inhibiting T cell cytotoxic response.[20]

On the other hand, escape may result from the development of an immunosuppressive state by the tumour's own microenvironment, by producing immunosuppressive cytokines such as vascular endothelial growth factor (VEGF), transforming growth factor–beta (TGF-β), IL-10, galectin, or indolamine 2,3-dioxygenase (IDO) and/or by recruiting regulatory immune cells that promote immunosuppression.[9,14,20,23] Regulatory T cells express inhibitory receptors such as PD-1 (programmed cell death protein 1), CTLA-4 (cytotoxic T lymphocyte-associated protein 4), Tim-3 (T cell immunoglobulin and mucin domain-3) and LAG 3 (Lymphocyte activating gene-3), with key roles in inhibiting host-protective anti-tumour responses therefore favouring tumour outgrowth.

2. TARGETED IMMUNOTHERAPIES UNDERLYING CANCER IMMUNOEDITING

Knowing the immune system's dual capacity to recognize and destroy cancer but also to shape cancer immunogenicity, consistent insights to control cancer via immunological means are now well establish. Currently it is widely accepted that CTLA-4, PD-1 and its ligand PDL-1 block T cell mediated immune system at different levels[24] and are important to control peripheral T cell response to preclude needless activation against self-tissues, but may also prevent destruction

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of tumour cells.[24] Therefore, immunotherapy with different agents intended to modulate the host immunity against the tumour, namely Interleucin-2 (IL-2), Interferon-α (INF-α), vaccines and immune checkpoint inhibitors targeting CTLA-4 and PD-1/PDL-1 are of utmost importance in the treatment of advanced melanoma (unresectable or metastatic) and also in an adjuvant setting.[25,26]

2.1 - Mechanisms of action of immune checkpoint inhibitors

2.1.1 – The cytotoxic T lymphocyte-associated protein 4 (CTLA-4)

CTLA-4 was originally isolated from cDNA mouse libraries. It was expressed in activated lymphocytes and co-induced with T cell mediated cytotoxity, hence the name "cytotoxic T lymphocyte-associated protein 4".[27] CTLA-4 is a type I transmembrane protein from the immunoglobulin superfamily, which also includes MHC class I and II molecules and the T cell receptor (TCR). Like other immunoglobulin family members, CTLA-4 is expressed on lymphocytes and takes part in the process of antigen recognition (35). Peripheral T cell response is controlled by two opposing costimulatory receptors (CD28 and CTLA-4) that bind to the same ligands (CD80 and CD86) on antigen-presenting cells (APCs) and induce a positive or negative feedback for T cell activation, respectively.[28,29] First, APC activate naïve T cells through MHC class I/TCR binding in the presence of co-stimulatory connection of CD28 with CD80 and CD86. Following T cell activation, CTLA-4 is upregulated and competes with CD28 for CD80/CD86 binding, thus attenuating positive co-stimulation by CD28.[30,31] Moreover, CTLA-4 has a higher affinity for the APC´s costimulatory receptors CD80/CD86 than CD28, and therefore disrupts the inicial binding[29] and T cells fall into an unrresponsive "anergic" state refractory to further stimulation.[32,33] In cancer, the main immune strategies for tumour escape may include the inhability of mutated tumour cells to develop a strong agonistic peptide epitope for T cells and, instead of a protective T cell response through CD28/CD80 or 86 ligation, a tolerogenic response may occur trhrough the CTLA-4 ligation. Several data have shown that enhancement of tumour recognition by positive T cell costimulation is beneficial.[32-34]

Despite its expression by activated CD8+ effector T cells, the major role of CTLA-4 seems to be on CD4+ T cells, downmodulating T cell helper activity and increasing regulatory T cell (T reg) immunosupressive function.[34] CTLA-4 insufficiency is a well established genetic factor for autoimmunity, suggesting the important role of this protein in regulating self-tolerance.[35,36] In cancer, CTLA-4 blockade promotes anti-tumor immunity[37] also directly increasing effector CD8+T cells and indirectly amplifying immune response by activating helper T cells.[35,38]

2.1.2 – The programed cell death1 (PD-1) and the PD ligand 1 (PDL-1)

PD-1 protein is another T-cell co-inhibitory receptor. Its structure resembles that of CTLA-4, but has different biologic functions and ligands.[39,40] PD-1 is expressed by chronically stimulated CD4+ and CD8+ T cells and also by B cells and natural killer (NK) cells.[41] The major role of PD-1 is to limit the activity of T cells in peripheral tissues during an inflammatory response to foreign as well as self-antigens, in order to regulate immune response and limit autoimmunity.[42,43] PD-1 is highly expressed in tumour-infiltrating lymphocytes, namely in melanoma, perhaps reflecting a major immune resistance mechanism within the tumour microenvironment.[44] PD-1 expression allows tumour growth despite the presence of tumour antigen–specific T cells in the tumour stroma.[45] Within the tumour bed microenvironement PD-1 predominantly regulates activity of effector CD8+T cell that display an exhausted phenotype and impaired effector function.[44]

The two ligands for PD-1 are PDL-1 and PDL-2.[43] The former is highly upregulated on the surface of many solid tumour cells, including melanoma.[44] PDL-2, has been less focused, and its expression is restricted mainly to macrophages, and also on dendritic and B cells.[46] Expression of PD-1 ligands is of outmost importance to determine suitability for a therapeutic blockade, because PD-1 only inhibits lymphocyte function when it is engaged by its ligands. Two mechanisms have been proposed for PDL-1 regulation within the tumour microenvironment: innate and adaptive immune resistance. In some tumours, PDL-1 expression is driven by oncogenic signalling pathway within tumour cells (innate immune resistance).[43] In other tumours, such as melanoma, PDL-1 is adaptively induced as a consequence of immune responses within the tumour microenvironment.[43,47] Specifically in melanoma, an interesting study[48] showed a strong correlation between PDL-1 expression on tumour cells and both lymphocytic infiltration and intra-tumor INF expression. Active antitumor immune response secretes cytokines such INF-γ that up-regulate PDL-1 expression. Thus PD-1/PDL-1 pathway induction may represent an adaptive immune resistance mechanism developed by melanoma cells in response to endogenous antitumor activity and may explain how melanomas escape immune destruction despite endogenous antitumor immune responses.

Taking into account all the previous findings it is quite reasonable that PD-1/PDL-1 pathway blockade will enhance host immune response against melanoma.[49]

CONCLUSION

Most cancer cells suffer an enormous number of genetic and epigenetic changes that confer plenty of tumour associated antigens that the host immune system can recognize, but many tumor cells escape immune destruction.

The cancer immunoediting concept advocates the integration of the effects of the host immune system on tumour development and outgrowth. The knowledge of the mechanisms underlying the phases of elimination, equilibrium and escape of cancer immunoediting are crucial for the development of new cancer immunotherapies. New insights concerning specific immune resistance mechanisms developed by tumours ("immune checkpoints") decrease antitumour

immunity and have a well-established role in the escape mechanism. CTLA-4 and PD-1/PDL-1 are the immune checkpoints whose mechanisms of action have been extensively studied and are currently applied on clinical treatment of several tumours.

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