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Influence of Anti PD-1 Therapy on Cancer Metastasis by Modulation of the Cancer Micro Environment

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Abstract: ***One of the major factors that make cancer particularly lethal is its ability to metastasize. Cancer, localised at primary site, is generally associated with good prognosis, however, its escape from the confines of primary organ and onto distant organs is largely untreatable (Zubair & Ahmad, 2017) . Major advances in cancer immunotherapy have dramatically expanded the potential to manipulate immune cells in cancer patients with metastatic disease to counteract cancer spread and extend patient lifespan. One of the most successful types of immunotherapy is the immune checkpoint inhibitors, such as anti-CTLA-4 and anti-PD-1, that keep antitumor T cells active. However, not every patient with metastatic disease benefits from this class of drugs and patients often develop resistance to these therapies over time (Edwards et al., 2020). This report focuses on the impact of anti PD-1 therapy on individual immune cells in order to determine its effect on the cancer microenvironment and the rate of metastasis. There are multiple different immune cells having various contributions towards metastatic growth and each of these cell types respond differently to Anti PD-1 blockade. It is therefore essential to understand immune composition and the driving factors of metastasis on a patent-specific level before recommending Anti PD-1 therapy. This will lead to a maximised potential of a positive clinical outcome, reducing the risk of cancer remission and metastasis.***

Keywords: cancer, metastasis, immunotherapy, immune cells, anti PD-1 therapy

1. Introduction

Genetic determinants of cancer

Cancer is a genetic disease in which some of the body's cells grow and divide uncontrollably. The three main genes responsible for cancer formation are proto-oncogenes, tumour suppressor genes and DNA repair genes. Proto-oncogenes are found in normal cells and their function is to stimulate cell division. Due to a gain of function mutation, these proto-oncogenes can convert to oncogenes. Oncogenes promote cancer by enabling uncontrolled accelerated cell division and an immortal phenotype. This in turn leads to increased cell proliferation, which is one of the hallmarks of cancer. Mutations in the tumour suppressor genes are also one of the main causatives in enabling cancer development. Tumour suppressor genes are responsible for slowing down cell division, repairing DNA mistakes and communicating apoptosis. Once this gene is inactivated due to loss-of-function or deletion mutation it leads to uncontrolled cell division and the formation of cancer.. In diploid organisms both alleles code for tumour suppressor proteins. If one gene is inactivated, the other allele continues to code for tumour suppressor proteins and hence the cell continues to function normally (*What Is Cancer? - NCI*, 2021). In case of both alleles being deactivated there is no tumour suppressor protein, leading to a cell being cancerous, a process summarised as the 'two-hit hypothesis. An example of this is a loss of function mutation in the p53 gene. The cells are no longer able to repair their DNA mistakes and can not undergo apoptosis, hence leading to the formation of cancer cells. DNA repair genes are the third main gene responsible for cancer formation. They are responsible for fixing damaged DNA and a mutation in these genes tends to lead to further mutations, such as duplication or deletion of chromosome parts (Henrik's lab, 2019,). As proto oncogenes and tumour suppressor genes allow the cell to grow and divide uncontrollably, DNA repair genes enable mutation in these cells. The body normally eliminates such cells with damaged DNA before they turn cancerous, but the body's ability to do so goes down as we age. This gradual accumulation in both pathways over long periods of time causes cells to become cancerous. (*What Is Cancer? - NCI*, 2021).

Hallmarks of cancer

Once these genetic mutations have occurred, there are certain traits that allow a cell to be cancerous. These traits are also known as the hallmarks of cancer (figure 1) and they are responsible for enabling cancer growth and metastatic dissemination. This paper will focus on "Avoiding immune destruction" as one of the major hallmarks of cancer (Hanahan & Weinberg, 2011, pp. 646-658).

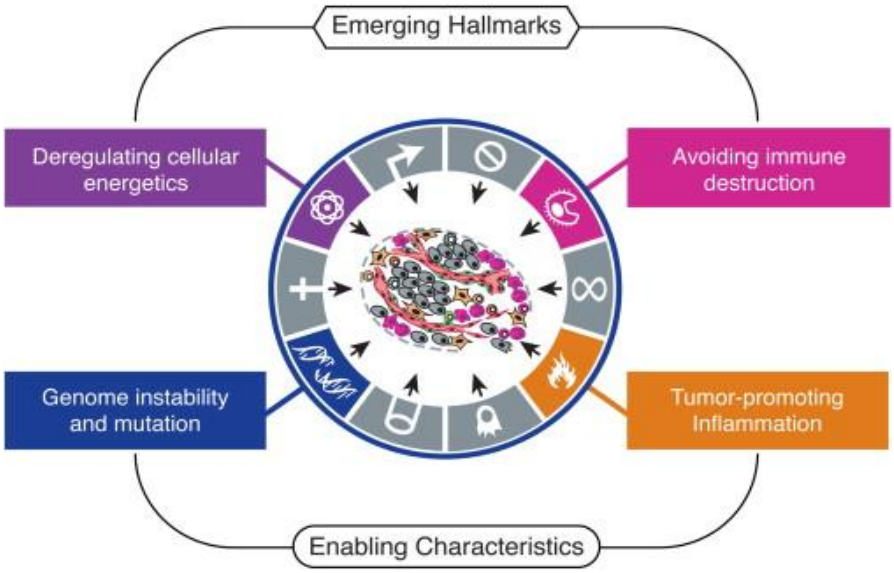

Figure 1: Hallmarks of cancer. Emerging hallmarks and Enabling characteristics of cancer.(reference – figure taken from Weinburg & Hannan 2010)

Cancer immunity cycle

In order for an anti cancer immune response to occur a series of stepwise events must be initiated and allowed to proceed. This is called the cancer immunity cycle. The first step in this cycle is the release of cancer cell specific antigens, known as neoantigens, to the surrounding environment. Neoantigens are the mutated proteins that are recognised by the immune system as 'non-self'. These released neoantigens are picked up by dendritic cells and other antigen presenting cells and migrated to the lymph nodes where they are presented on MHCI and MHCII molecules to the resident T cells. Upon specific recognition of the cancer specific antigens that are viewed as foreign by the T cell receptor (TCR) of a T cell results in its activation and hyper proliferation. In this stage it is important for the ratio of T effector cells to be greater than T regulatory cells, to allow for an effective immune response and this ratio is often determined by how the neoantigen is recognised by the T cell repertoire. If there are more antigens specific to the production of T effector cells present, in comparison to the antigens of T regulatory cells, a greater ratio of effector:regulatory cells are produced, making the immune response more effective. After the production of effector T cells, these T cells migrate to and infiltrate the cancer bed down the chemokine gradients. These cytotoxic T cells then recognize and bind to the cancer cells through interactions between T cell receptors and its respective antigen. Along with this, a series of co-stimulatory signals are also required; such as initial T cell activation provided by the cancer cells, neighbouring cells and multiple environmental factors. This interaction results in induced apoptosis and the killing of these cancer cells. This in turn results in an increased production of tumour-associated antigens, hence broadening and strengthening the immune response against cancer (Chen & Mellman, 2013, pp. 1-2).

Immune checkpoint blockade

Immunoediting is a dynamic process that consists of immunosurveillance and tumour progression. It consists of Elimination, Equilibrium and Escape. Elimination occurs before there is clinical evidence of tumour formation and during this stage the immune system is effective in battling cancer cells. After Elimination, the immune system and cancer cells reach an equilibrium. During this stage the cancer cell growth remains dormant while they develop more mutations. Simultaneously immune cells are being recruited to eliminate cells that are identified as foreign, creating a selective pressure on tumour cells. This selective pressure results in tumours developing beyond the control of the immune system and this stage is known as Escape (Tharmalingam, 2016). One escape mechanism created by the tumour to evade effector T cells is to produce immune checkpoints such as PD-1 to inhibit T cell activity. Immune checkpoints are regulators in immune activation and they play a key role in maintaining immune homeostasis and preventing autoimmunity. An example of an immune checkpoint is PD-1, a receptor that is primarily expressed on mature T cells in peripheral tissues and the tumour microenvironment (Creative Biomart, 2018). PD-1 is responsible for regulating T effector cell activity within tissues and tumours. In order to evade immune surveillance, tumour cells express PD-L1 on their surface, which is a ligand for PD-1. This expression leads to a negative signal when PD-1 is bound to PD-L1, leading to suppressed T cell activity and hence an increased cancer growth rate. To combat immune escaped tumours, immune checkpoint inhibitors were developed. These inhibitors are responsible for blocking immune checkpoints and hence restoring the immune system to its full functioning. Anti PD-1 therapy is an example of immune checkpoint blockade. This therapy blocks PD-1 and its ligand (figure 2), leading to increased immune activity and in turn a stronger immune response to cancer (Lei et al., 2020). While some patients respond positively to this treatment, it results in an increased rate of cancer growth for others. This paper will look into the effects of anti PD-1 therapy on the critical elements of the cancer microenvironment and in turn its effect on the rate of metastasis.

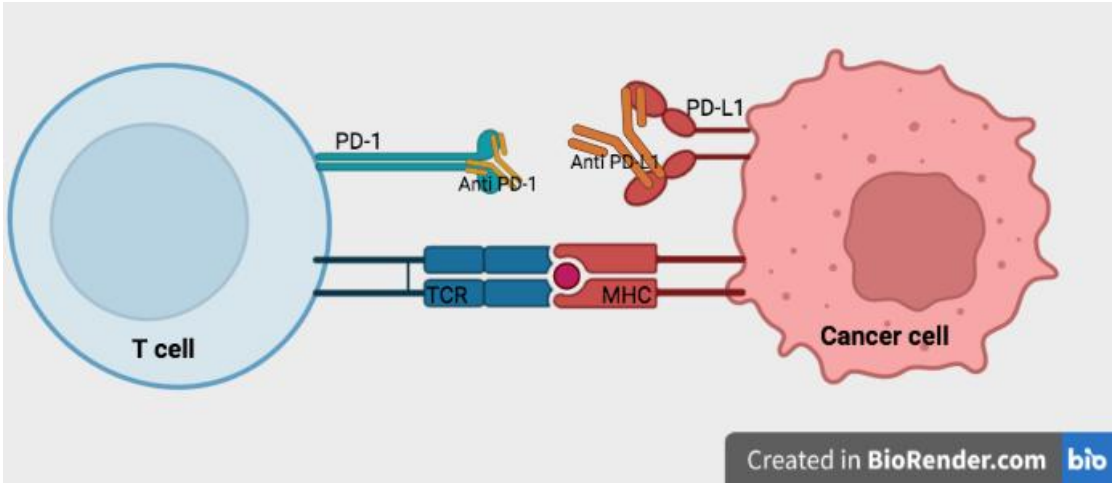

Figure 2: Anti PD-1 therapy mechanism. Created with BioRender.com

Cancer Metastasis

Cancer metastasis is the spread of tumour to sights that are physically discontinuous from the primary tumour. It is the major cause for cancer related deaths. In order for metastasis to occur, there must be a series of genetic and epigenetic alterations in some cells. These alterations drive what is known as the metastatic phenotype and can be a consequence of factors such an acidic environment and lack of oxygen at the primary site. These changes in the cell microenvironment eventually leads to a metastatic cascade (figure 3). This process begins when cancer cells move forward to the basement membrane by rearranging their cytoskeleton and attaching to the extracellular matrix via proteins outside their plasma membrane . Once the tumour cells reach the basement membrane, they secrete various proteolytic enzymes that help break down the basement membrane and enable the tumour to penetrate the blood vessels. These cancer cells now start a multistep process of locomotion which includes penetration into the endothelial basement membrane and transmigration into the vascular spaces. Inside the blood vessels, the tumour migrates in the form of multicellular aggregates , some of these cells containing stem cell like properties to help the tumour adapt to a new environment. The tumour cells then deposit to a site based on the seed soil hypothesis or other explanations such as due to mechanical factors. This means that these tumour cells deposit and grow at a site favourable to their growth (ilovepathology, 2021).

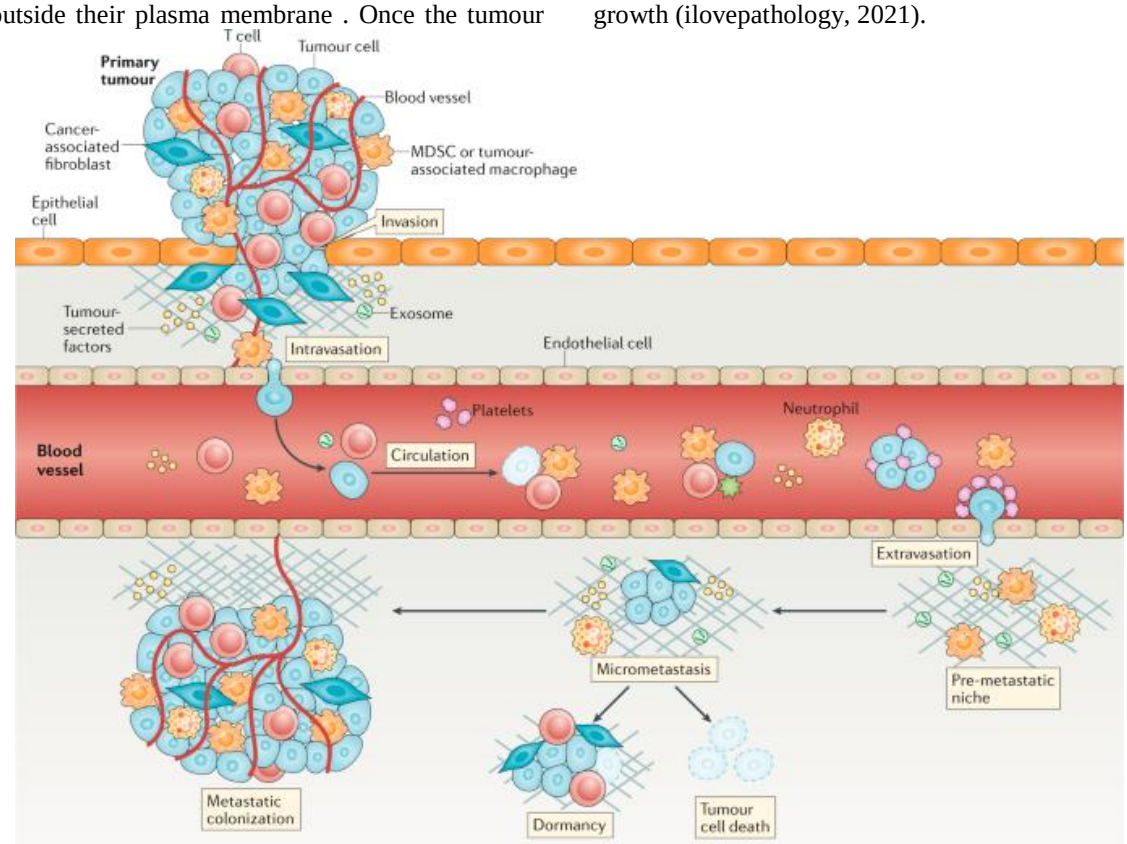

Figure 3: Overview of metastasis.(figure taken from Cancer Research UK and Cancer Therapeutics CRC Australia Metastasis Working Group 2019)

Cancer microenvironment

The tumour microenvironment consists of a complex network of different cell types and structures that surround and interact with the tumour cells. Cells within the tumour microenvironment consists primarily of cancer cells, a wide range of immune cells and structural support cells such as stromal cells. The tumour microenvironment has certain features which enables it to allow continued survival and growth of the cancer cells. One of these features is immunosuppression;for example, creation of immune checkpoints, a reduced release of tumour antigen into the draining lymph and recruitment of regulatory T cells. The tumour microenvironment is also a metabolically demanding environment and this competition for oxygen and other nutrients often leads to the tumour cells outcompeting weakened immune cells (Tokuyasu & Huang, 2018).

How does Anti PD-1 therapy influence immune cells and their effect on cancer metastasis

CD8 T cells

CD8 T cells, also known as cytotoxic T lymphocytes, are an important part of the adaptive immune system. In a tumour microenvironment the CD8 T cells are a critical component in the immune system for recognizing and killing cancer cells. In order to carry out the process of recognition, CD8 T cells detect neoantigens presented on APCs (Antigen presenting cells) such as dendritic cells. Once a CD8 T cell recognizes a presented neoantigen through its complimentary T cell receptor (TCR), alongside a series co-stimulatory signal provided by the APC, these T cells are activated and are able to kill cancer cells through a variety of mechanisms including the direct induction of apoptosis. CD8 T cells play an important role in creating an anti-metastatic environment, by killing cancer prior to dissemination as well as killing cancer cells present in the circulation during dissemination (Janssen et al., 2017). They also display anti-metastatic activity by killing cancer cells upon their entry into the metastatic niche (an environment in a secondary organ that can be conducive to the metastasis of a primary tumour) (Janssen et al., 2017). PD1 is increasingly expressed on CD8 T cells as they become more active due to a protective negative feedback loop. Hence the functioning of CD8 T cells is directly impacted by anti- PD1 therapy. CD8 T cells are anti-metastatic and this activity is increased due to PD-1 blockade. However it is important to note that if there are insufficient CD8 T cells to destroy the tumour, the T cells will succumb to exhaustion and the treatment will have a negative impact on the patient (Zheng et al., 2019).

CD4 regulatory T cells

CD4 regulatory T cells are a critical component of maintaining immune cell homeostasis and preventing autoimmunity. However this immunosuppressive activity of these T regulatory cells can often lead to increased tumour growth and metastasis. T regulatory cells are often pro-metastatic and their recruitment in the primary tumour is often necessary for metastasis. Hence, by producing immunosuppressive cytokines, the tumour promotes CD4 regulatory T cell proliferation over other antitumor T cell subsets and the tumour environment can also promote 'Induced regulatory T cells' (iTREGs). T regulatory cells promote a pro-metastatic environment by inhibiting the differentiation and proliferation of cancer-killing CD8 T cells in the primary tumour as well as limiting their function in the circulation. They can also inhibit dendritic cell functioning and maturation, further contributing to an increased rate of metastasis (Janssen et al., 2017) . PD-1 receptors are expressed on CD4 regulatory T cells. This means that during Anti PD-1 therapy, the activity of these pro-metastatic cells is increased, which can increase the rate of cancer metastasis.

Macrophages

Macrophages are specialised cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms. They are also responsible for acting as APCs for T cells and initiating inflammation by releasing cytokines. In a tumour microenvironment there are two different types of tumour associated macrophages, namely TAM1 macrophages and TAM2 macrophages. TAM1 macrophages are inflammatory and are tumour suppressive. They also stimulate the immune system to attack the metastatic cancer cells throughout the metastatic cascade. TAM2 macrophages decrease CD8 T cell infiltration and are protumorigenic (Janssen et al., 2017). TAM2 macrophages also prove to be pro-metastatic by promoting angiogenesis and hence increasing the probability of tumour migration. Once tumour migration starts these macrophages promote metastasis by shielding the metastatic tumour from the immune system. In the final stage of metastasis, TAM2 macrophages promote secondary tumour growth by inducing an immune-suppressive environment (Janssen et al., 2017). Anti PD-1 therapy has shown to increase the number of TAM1 macrophages while decreasing TAM2 macrophage population. This means that Anti PD-1 therapy will promote an anti-metastatic immune response through macrophage activity (Xiong et al., 2019).

2. Concluding Remarks

Patients respond differently to Anti PD-1 blockade; driving tumour remission, being ineffectual or paradoxically accelerating tumour growth and metastasis. As described in the report, there are multiple different immune cells having various contributions towards metastatic growth and each of these cell types respond differently to Anti PD-1 blockade. It is therefore essential to understand immune composition and the driving factors of metastasis on a patient-specific level before recommending Anti PD-1 therapy. This will lead to a maximised potential of a positive clinical outcome, reducing the risk of cancer remission and metastasis. It will also have fewer side effects since these personalised treatments are more likely to target cancer cells over healthy cells. Patient specific treatment can be carried out by identifying biomarkers and carrying out gene sequencing since many cancers involve specific genes being altered. Working towards building these personalised cancer treatment plans would include screening tests, genetic tests and predicting the risk of recurrence. These techniques can help determine the correct treatment option and accurately evaluate patient progress during treatment. Examples of personalised treatments that are currently in practice include targeted therapy and pharmacogenomics (study of how our genes impact drug response). Although these personalised treatments have started to be implemented in cancer

diagnosis, they are not available for all types of cancer and also prove to be expensive and only available during clinical trials (Cancer.Net, 2020). Hence to maximise positive treatment outcome through immunotherapy, patient-specific cancer treatment needs to be fully developed.

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