Limitations of Adaptive Immune System in Solid Tumours

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Keywords: Solid tumour, adaptive immune, immunotherapy, cancer

Abstract: TGF-beta, as a transcriptional factor can regulate the populations of different type of immune cells. Via activation several biochemical cascade (P38/JNK, AKT, ERK), more Treg cells, which act as immune suppressors, are expressed at a higher level; and the cytotoxic immune cells apoptosis also happens. Consistent with the effects of Treg cells, both CD4+ and CD8+ cells which express dominant-negative form of TGF-beta receptor have been reported to have stronger cancer immunity. Hypoxia, on the other hand, is another typical condition of solid tumours. As the cytotoxic immune cells usually depends on both glucose and oxygen to function, the immunity of such cells is usually weakened under hypoxia. Also, hypoxia can let the immune cells down regulate cytotoxic enzyme. Lactates over production can be a consequence of hypoxia, nevertheless, it impacts the immune cells' function through way. Besides expression of Treg cells, it can also down regulate the glycolysis of CD8+ cells. The capability of CD8+ cells to proliferate and produce cytokine are weakened. Furthermore, the macrophages are affected and shift to M2 state in presence of highly concentrated lactate, and M2 activated macrophage can confer the tumour cells extra immune resisitence. This paper aims to revisit and summary the studies which review the mechanism underlying the limitations of the adaptive system in solid tumour. There are three major aspects that been covered by this paper: TGF-beta, hypoxia, and lactate.

1. Introduction

For the past several decades, the development of biochemistry allowed us to use more advanced technique to fight against cancer. One evolutionary outcome is the adoptive cell therapy of cancer. Adoptive cell therapy is modified human immune cell which can be used to get clear of cancer cells. In comparison with the traditional chemotherapies or radiotherapies, the major advantages of adoptive cell therapies are higher specificity and less tissue damaging. T cells play a very important role in adoptive cell therapies as they cross link different immune response, activated T cells can elicit and facilitate innate and adaptive immune system at the same time.

TIR is one genetically engineered T cells adoptive cell therapy that has been developed in past decades, and the T cell source is tumor infiltrating lymphocytes which includes T cells and peripheral blood mononuclear cells. Such cells are used as the local immune cell at tumour sites tends to have tumor-target specificity. Clinically, the isolated lymphocytes are cultivated and risen to more versatile population of immune cells using different cytokines. Chimeric antigen receptor (CAR) T cell technique is another way to eliminate tumor cells depending on patients' immune systems. The CAR is inserted to the T cell surfaces to enhance the specific immune response to tumor cells. In comparison to human T cell, CAR T cell de not relies on the MHC to initiate an immune response [1]. Up to now, there are three generations of CAR T cell and for each generation, extra domains are added to the receptors embedded. Research shows the CAR T cell developed in proliferation, persistent and cytotoxicity throughout three generations. However, the modified immune cells process the same metabolism as the normal immune cells, which is consistent with the adoptive cell therapies are more limited in solid tumors rather than in hematological tumors. Such suppressions can be due to several factors including poor penetrations to tumor sites. Thus, solid tumours and their complex conditions within them are challengeable to the new developed adoptive cell technique. Thus, the CAR T cell

technique has a better efficacy in treating haematogenic tumour than it does in solid tumour, and that is the reason for that CAR T cell is only opened for one kind of amyloid treatment.

In cellular molecular level, the suppression can be caused by inhibitory cells accumulations, insufficient inflammatory factors, and tumor secreted inhibitory proteins. Due to the influence of such factors, the T cells' tumour targeting clearing capability can be down regulated, which usually leads to low efficacy of immune therapies.

This paper aims to summarize the impacts of ordinary factors associate with solid tumours like TGF-beta, hypoxia and lactate on the adaptive immune systems. Such factors usually associate with others and impact the cells on both metabolic pressure and transcriptional level, and result in weak cytotoxicity and overpopulated pro-tumour cells, which is a huge challenge for immune therapies.

2. The CAR T cell has limitations in solid tumors clinical treatment

Despite the CAR T cells therapy is a highly feasible way to fight against cancer; and 2 drugs have been approved by FDA in 2017, such therapy is not as competitive as it does in hematological tumors. For example, according to A phase II clinical trial in 2017, among 111 B cell lymphoma patients have taken the anti CD19 CAR T cell therapy, the objective response rate and complete response rate achieved 82% and 54%, respectively. However, in another study, none of 10 anti-EGFRvIII CAR-T cells treated neuroblastoma patient showed any positive response. The unsatisfied result is very likely due to the highly limited interaction between T cells and solid tumors which lack of adhesion molecules and normal vessel constructions. Also, it is suggested that both the proliferating capabilities and the tumor specific toxicity of CAR T cells can be impaired in the tumor microenvironments.

The tumor microenvironment (TME) is a critical character of the solid tumor. TME is a complex environment consists of immunocytes, fibroblasts, extracellular matrix and signaling molecules, and most of them are abnormal. In such environment, there are a series of molecules can lead to immune resistance of tumors. For example, the TGF-beta in TME is thought to be associate with low immunity to tumors. However, the TGF-beta mostly comes from the immune system itself but not the tumor.

3. TGF-beta down regulate the immune cell response in transcriptional level

Transforming growth factor beta (TGF-beta) is a classic membrane to nucleus pathway, which inhibits expansion and function of several parts of immune system. In the pre-maglignant state, the TGF-beta can inhibit tumour genic inflammation and limit stromal-derived mitogens' level. However, in malignant progression, the TGF-beta can inhibit the immune system via several ways. Thus, the tumour progression and metastasis can be enhanced by TGF-beta. Some studies have suggested TGF-beta in tumour site can increase the cytotoxic T-cell (CTL) and tumour infiltrating lymphocytes (TIL) surveillances that improve prognosis [3,4,5]. Also, a study revealed the blocked TGF-beta signaling leads to increased presence of CTL at tumor site; and both endogenous and exogenous TGF-beta has such inhibitory activity on CD8+ T cell [6]. Similarly, both CD4+ and CD8+ cells which express dominant-negative form of TGF-beta receptor and such cells are suggested to have stronger anti-tumour immunity [7,8]. Recent studies also found that TGF-beta also induce the short-live CD8+ T cell's apoptosis, such kind of CTL contributes to over 90% effector cell pool in certain circumstances [10].

Treg cell can inhibit the cytotoxic cell and they are affected by TGF-beta. *FOXP3* gene plays an important role in the conversion from CD4+ cells to Treg cell. Forkhead box P3 (*FOXP3*) is a transcription factor which down regulates the immune activity of certain immune cells to prevent the autoimmunity [17], and CD4+/CD25+/*FOXP3*+ cells are known as the autoimmune suppressor [18-20]. In early studies, several observations indicated the potential impacts of TGF-beta on Treg cells: pancreas cancer patients are immunosuppressed and they have both chronically elevated serum TGF-beta and Treg cells [11, 12]. In the tumour microenvironment, TGF-beta can both recruit natural T regulatory cells and promote the conversion from CD4+ T cells to Treg [13]. Moreover, such induced Treg has more efficacy in inhibiting CTLs' activity than natural Treg cells derived thymically [14, 15].

A TGF-beta cascade is launched as such Tregs presents TGF-beta on their cell surfaces and the anti-tumour response are attenuated through a cell-to-cell contact manner. Although the direct CD8+ suppression mechanism is unknown, the impact of TGF-beta on innate immune systems via Treg cells includes decreased interferon-gamma, less efficient effector cell recruitment, and lowered cytotoxic activity [14,15]. On the other hand, recruitment of Treg cells from systemic circulation to local tumour sites was observed in an ovarian cancer model, in a CCL22 dependent way (a Treg specific chemokine) [16]. However, not all the Treg cells express *FOXP3* and CD25. There is a study suggests that the TGF-beta converts CD4+ T cell to Treg does not depend on the presence of CD25 [21]. On the other hand, a new kind of Treg cells have been identified, which express no CD25 or *FOXP3*; this kind of Treg cells is reported to be capable to express TGF-beta on their membranes in case the CD69 receptors are activated and the production of TGF-beta may also leads to the promotion of tumour development [22]. Alike to CD4+ Treg cells, CD8+ Treg cells also have immune suppressing activities. In a study, TGF-beta and IL-6 co-induced CD8+ Treg cells was observed to have ex vivo suppressing activity [23]. However, the CD8+ Treg cells has a much smaller number than CD4+ Treg cell.

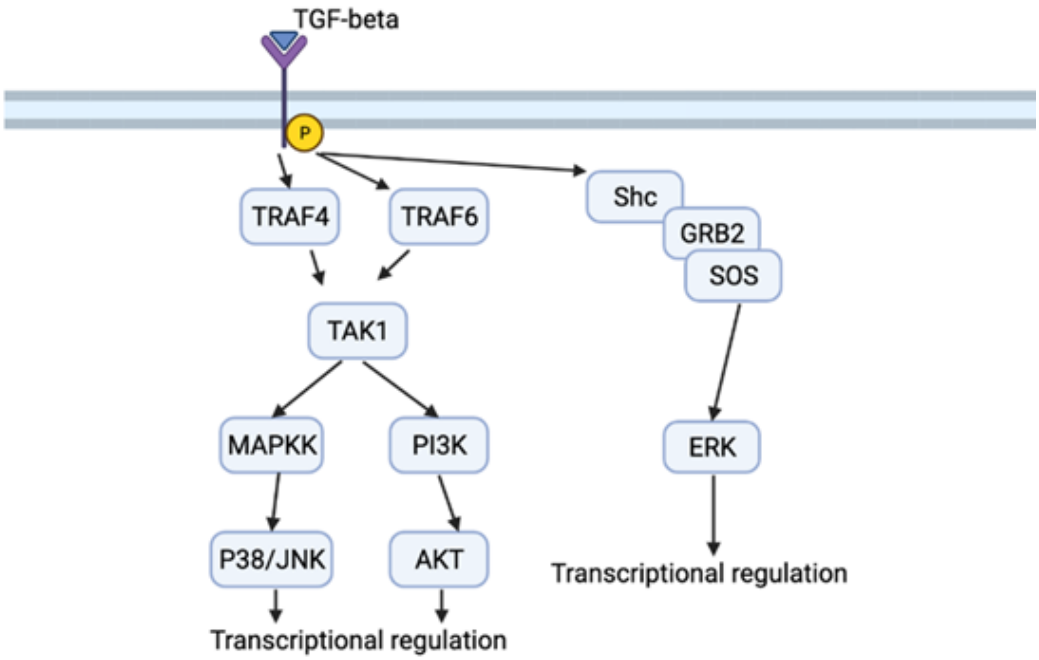

Figure 1. The signaling pathway of TGF-beta [57] (The TGF-beta activated receptor can launch several biochemical cascades. The figure shows the biochemical pathways that lead to transcriptional regulations which cause the proliferation, differentiations, apoptosis of the T cells.)

4. Hypoxia associate with the solid tumour and its impact on adaptive immune system

Hypoxia is another key factor of tumour microenvironment and the cell in such environment experience not only the low oxygen tension but also the HIF (hypoxia inducible factor) pathway activation. An in vitro studied discovered that T cells' HIF pathway is stabilized even in atmosphere oxygen level [24-27]. A study group test the CTL cell under hypoxia and normoxia condition, surprisingly, the oxygen content was found to have no effect on CTLs' cytotoxic activity [28, 29]. However, hypoxia can induce autophagy via STAT3 pathway and miR-210 up-regualtion and finally cause degradation of granzyme B which plays an important role in tumour cell lysis [31-34].

Consequently, hypoxia can confer enhanced cytotoxic resistance against the CTLs of tumour cells [30]. Despite the cytotoxic activity of the CTLs maintain the same under hypoxia condition, the capability to proliferate and viability of CTLs can be weakened. The positive correlation between the oxygen levels and the expansion and viability of the CTLs is suggested [35]. However, the mechanism maintains unknow and several possible factors are proposed. The local ATP level is usually related to the glucose and the oxygen availability, the decreased ATP level can lead to the lower viability of the

CTL cells. Nevertheless, such propose is not possible as there are studies suggested T cells can maintain the ATP level as the same even under hypoxia [36-38]. On the other hand, there are controversies on whether the HIF-1a can regulate the mature T cell expensions [39, 40] After the text edit has been completed, the paper is ready for the template. Duplicate the template file by using the Save As command, and use the naming conly created file, highlight all of the contents and import your prepared text file. You are now ready to style your paper; use the scroll down window on the left of the MS Word Formatting toolbar.

Also, although the decreased level of cytokine IL-2 is observed, and IL-2R-alpha is instead secreted, such feature does not affect the CTL's expansion [41, 42]. In contrast, there are studies suggest that hypoxia can be helpful to the CD8+ cytotoxic cells' anti-tumour function in certain context. One study described an HIF-dependent increased cell killing capacity of T cells in a chronic infection or in a tumor context, and this is because of the HIF's regulation of the CTL priming [43]. However, as the study is not carried out under hypoxic condition, the mechanism can be deduced to be not dependent on the HIF only, and only HIF-2a level are increased under hypoxia condition [44]. Besides the different abilities to expansion, the capacity to secrete cytokines are also affected by hypoxia. Enhanced IL-10, IL-2Rα, and 4-1BB expression in reactivated CTL under hypoxia condition are observed. Such feature can be a launch points for the further studies of immunotherapies [45-47].

5. Lactate and metabolisms of the immune cells in cancer treatment

High local lactate level is another feature of tumour microenvironment which usually exploited by the tumour cells to invade the immune system, and this is because the tumour cells mainly depend on the glycolysis to gain energy and OXPHOS pathways are usually impaired. The lactate level in tumour site can rise up to 40 folds [48-50]. Lactate can inhibit the function of both innate and adaptive immune cells and this review focuses on the adaptive parts of the immune system. One of the mechanisms is that the lactate can interact with sodium ions to form sodium lactate. Such chemical can bind to transporter SLC5A12 which locate on the CD4+ T cells' surface which leads to mis-migration, up-regulated cytokine IL-17 production and down regulated glycolytic enzymes [51]. The combined three factors can cause chronic inflammations which may leads to more beneficial factors for tumour cells. On the other hand, a study using HCl to investigate the influence of the low PH to the CTL cell and it showed that low pH leads to the impaired CTL proliferation, cytotoxicity and cytokine production [52, 53]. The cytokine production can be decreased by up to 95% and the number for cytotoxicity can be up to 50% [51]. Unlike to hypoxia, lactate impair the cytokine production in a different way. The IFN-γ production is blocked as the TCR-triggered phosphorylation, p38 pathways, and activation of JNK/c-Jun does not function well in presence of lactate [55]. Also, the blocked exportation of the lactate to the CTL cells disrupt their metabolisms as the concentration gradient is heavily imbalanced in the TME condition [54]. Moreover, the tumour cell derived lactate can leads to downregulation of the expression of FAK family-interacting protein FIP200, the apoptosis of the naïve T cell, and impairment of autophagy (the treatment target of autophagy in cancer context is controversial) [57]. Also, the Treg cells accumulate at the tumour site with the high concentration of lactate. The Treg cells' FoxP3 pathway is upregulated due to the high lactate concentration and mitochondria of such cells are highly activated, such features confers the metabolic advantage of Treg cells at tumour site and cause Treg cell accumulation. Such adaptivity of Treg cells at tumour site enhance their immune suppression abilities 33.

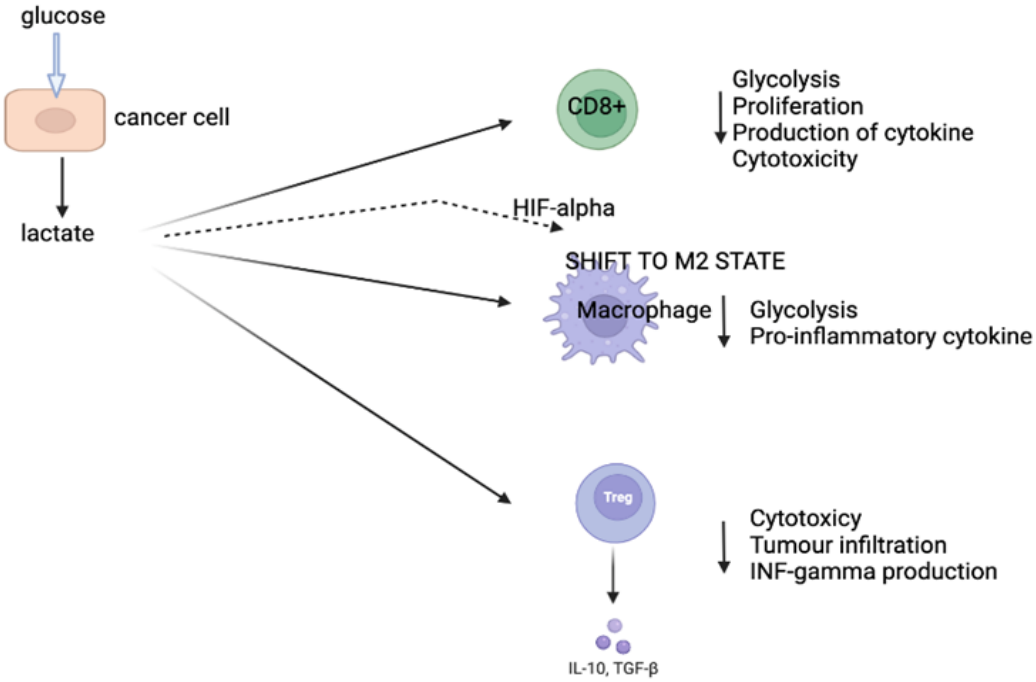

Figure 2. Lactate impact on major immune cells at tumour site [56] (Lactate has profound impacts on different immune cells. The lactate production is highly related to the hypoxia condition in solid tumour. Lactate can down regulate the proliferation, production of cytokine, glycolysis, and cytotoxicity of CD8+ T cells. Similar impact is also observed on Treg cells. Also, lactate combined with hypoxia lead the macrophage shift to M2 state, which is responsible for the tissue maintenance and repair. All the impacts can be assumed as failure in immune response against tumours.)

6. Conclusion

The solid tumour associate with the TME are now vital in the immune therapies. The local chemicals usually disrupt the function of the CTL cells. The TGF-beta promote the conversion of the naïve T cells to the pro-tumour T cells and weaken the cytotoxicity of the CTL cells. This study claims the blocked TGF-beta pathway can suppress the cancer progression [56]. Hypoxia can also lead the weakened immune response in a different way. The cytotoxic enzyme concentration is decreased. The expansion and the reactivation are also inhibited by such condition. Although hypoxia can be a target for cancer treating, it is usually focused on the decreased survival of hypoxic cells, which cannot be used together with the immune therapies. The lactate suppress the immune response via impairment of the CTL cells' metabolism and the Treg cells. However, such effect can be abrogated by increasing the local pH. Collectively, the CAR T cell immune therapy are facing challenge of TME and the cocktail therapies which block certain chemicals can be one of the future directions to provide the CAR T cell a suitable environment to function.

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