**Stimulation of immune responses and reduction in immunosuppression of tumor microenvironment by combination therapy**

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The tumor microenvironment (TME) has been recognized as a major obstacle to the use of immunotherapies to reduce cancer cells. As shown in past preclinical studies, therapeutic cancer vaccines have shown great promise in the treatment of solid tumors. However, the clinical effects have been indicated to be limited in cancer patients. It is thought that this effect is a result of the immunosuppressive capabilities and the prevention of the adequate infiltration of antitumor T cells in to the TME. However, recent studies have displayed the potential of combination therapies and their ability to induce an improved antitumor immune response. Therefore, it is important for the treatment of established tumors to develop combination therapies of vaccines with additional therapies, such as radiotherapy (RT) and PD-1 blockade. This review will look closer into the establishment of the immunosuppressive nature of tumor microenvironments, along with changes in immune cells in relation to monotherapy and combination therapy use. It will further discuss specific immune cells, including Tregs and effector cells and their involvement in the TME and tumor growth. Lastly, advances and limitations in this field of study of will be reviewed involving the use of combination therapies on the TME.

**Introduction**

Cancer is a pervasive disease, supported by a wide range of both malignant and benign cells within the human body that help facilitate tumor progression (Reviewed in Chen et al., 2015). This progression is induced by regulatory T cell (T-reg) recruitment into the tumor microenvironment (TME). It leads to immunosuppression that diminishes T cell immunity, resulting in an increase in tumor growth (Zhang et al., 2021).

Among T-regs, there are many different T cell populations present within the TME. For instance, the CD8+ T cells, which are typically associated with killing of tumor cells. These are typically supported by CD4+ T helper 1 (TH1) cells. Increased numbers of these cells correlate with a good prognosis. Similar to TH1 cells, the T-regs are a class of CD4+ T cells. However, they are tumor promoting (Reviewed in Balkwill et al., 2012; Reviewed Chen et al., 2015). Clinically, it was shown that in the presence of a higher ratio of CD8+ T cells to CD4+ T-regs, there was an association of better survival in all cancer types. This data suggests that the ratio CD8+ and T-regs could be correlated with tumor progression and cancer survival.

Currently, Radiotherapy (RT) is used as the standard treatment and is used on up to 50% of all cancer patients (Zhang et al., 2021). Recently, data has suggested that RT by itself rarely effectively activates antitumor immunity in cancer affected patients. This could be due to the fact RT alone cannot induce adequate peripheral effector immune cells and recruit them into irradiated tumor growths (Rücker et al., 2021; Zhang et al., 2021).

Cancer immunotherapy involves changing the immune system to allow it to focus on the identification and destruction of affected cells that could potentially form malignant growth (Xie et al., 2019; Tan et al., 2021). Immunotherapies have great potential to add to RT by boosting its ability to induce immune responses. As well as counteracting intrinsic tumor effects and RT facilitated immunosuppression (Rücker et al., 2021). Because of this potential, finding an optimal synergy between therapies is important in cancer treatment (Zhang et al, 2021).

**Recent Progress**

*Change in immune cell expression in irradiated primary and non-irradiated abscopal tumor sites.* Both primary and the non-irradiated abscopal tumors respond in a different way to immunotherapies. A recent study determined the immune cell composition differs between the two tumor sites. Primary tumors after treatment of RT displayed a higher concentration of CD45+ immune cells. Additionally, these tumors displayed higher concentrations of plasmacytoid dendritic cells (pDCs), dendritic cells (DCs), Natural killer cells (NKs), monocytes, and macrophages. Furthermore, by subdividing the macrophage/monocytes and DCs further, the data displayed that the CD11b- DC (cDC1) subtype percentage of DCs were significantly greater in primary tumors after treatment of RT but not abscopal tumors. The study’s data results also saw no significant changes in basophils, neutrophils, eosinophils, and B cells. Nevertheless, there was a decrease in these cell populations in primary tumors after irradiation of RT.

Additionally, the study displayed that there was a lower number of immune cells in irradiated tumors, as well as a decrease in the CD45+ immune cells and other cell types in peripheral blood after RT (Rückert et al., 2021).

*Host Immunosuppression by the progressive reduction of CD8+ T cells and increasing T-regs in tumor-bearing mice.*A recent study revealed that T-regs and Myeloid derived suppressor cells (MDSCs) contributed to immunosuppression and permitted inactivation of peripheral and tumor T cells, resulting in the escape of tumor immunity. The data from this study shows that tumor-bearing mice might play a role in affecting host immune response. However, when the mice were treated with RT, only the percentage of T-regs significantly increased in response. This would suggest that only T-regs has a predominate role in radiotherapy resistance. In addition, in non-tumor bearing mice, there was a significant reduction in the CD8+ T cells compared to the CD8+ T cell to T-reg ratio in tumor-bearing mice (Zhang et al., 2021).

*Increase in immunosuppression in the tumor microenvironment by radiation.* As previously displayed, irradiated tumors experience an increase in CD45+ immune cells. This is due to the irradiation causing an influx of leukocytes into the tumors (Rückert et al., 2021; Zhang et al., 2021).

Additionally, evidence displayed after radiation of the tumors, there was a significant increase in CD8+ T cells, but the overall percentage of the cells were low. On the other hand, T-regs were increased by 2.1-fold in the tumor when compared to non-irradiated tumors. The results suggest that this increase in T-regs contributes to irradiation stimulated immunosuppression in the models used in the study.

 Moreover, the evidence suggests a decrease in CD8+ T cells to T-regs plays a crucial role in adaptive immune response suppression within the TME. This is further explained by the data results. When CD8+ T cells are depleted, there is only a slight elimination of the RT induced effect. This is added to by the fact that when CD25+ T cells are depleted, this is enhanced. Though, when tumors are treated with only RT, tumor-bearing mice survival did not increase when they were treated with RT in the presence of CD25+ cell depletion. This data suggests that suppression of T-regs alone does not provide significant enhancement to antitumor immunity. For that to be achieved, it may require an increase in CD8+ T cells to T-regs.

*RT-mediated tumor control by combinations of RT and vaccination.* Vaccines have been shown to have potential to increase CD8+IFN-y+ T cells levels when it is administered before RT treatment. This results in increased antitumor activity. In a recent study, it was shown that RT when administered alone could only maintain tumor degeneration for up to 10 days before the tumor quickly relapsed. Therefore, the researchers tested mice with different treatments of RT and vaccine E7. After 14 days, they discovered the best tumor outcome was with the E7 + RT treatment. It displayed a significant decrease in tumor weight and interestingly a 4-fold increase in effector CD8+IFN-y+ T cells and CD45+CD8+ cells compared to the other treatments of RT+E7 and only RT.

 The study then determined the order of the therapies given affects the anti-tumor immune response. Two treatment groups were given RT and two doses of the E7 vaccine in opposite orders. The E7+RT group had significantly higher percentages of CD8+IFN-y+, CD8+ T cells, and CD45+ cells compared to the other treatment group. In addition, the ratio of CD8+ T cells to T-regs saw improvements resulting in a better immune response in those tumors. This suggests the order of the combination therapies affects the immune-mediated antitumor effects.

In the past, the clearance of solid tumors has been a challenge within immunocompetent mouse models. In addition, it has been shown that vaccinations by themselves cannot clear solid tumors above 100 mm3.

 In the study, they tested four treatment regimens: RT alone, vaccine alone, RT followed by E7 (RT+E7), and E7 followed by RT (E7+RT) on localized tumors above 100 mm3 in tumor-bearing mice. Although, both treatments alone delayed tumor growth for approximately 10 days, the tumors eventually began to regrow. RT+E7 displayed better delayed tumor growth when it was compared to RT alone. However, it did not exhibit strong tumor regression and a lasting cure. In contrast to the other treatments, E7+RT did exhibit strong tumor regression as well as a lasting cure in 44.4% of TC-1 tumor bearing mice (Zhang et al., 2021).

**Discussion**

It has been recognized that malignant cancer cells are not the only target of RT and chemotherapy. They are also known to affect the TME and their actions on the microenvironment impact whether the treatment fails or succeeds. It has been indicated that these treatments are most successful when they cause anti-tumor immune responses. The importance of our understanding of the induced immune responses in tumors by various therapies as well as how to incorporate this information to create an effective treatment regimen is the utmost importance (Reviewed in Balkwill et al., 2012).

*Anti-tumor immune responses and immune cell infiltration.* Despite the quantity of immune cells present in both primary and irradiated abscopal tumor sites, it does not always correlate to the amount of infiltration of immune cells after a particular treatment. During a study involving a melanoma model, it was discovered that despite the attraction of T cells due to a combined peptide vaccine and incomplete Freund’s adjuvant, the cells were only attracted to the injection site and not into the tumor site ensuing in impaired tumor control.

*Changes in immune cell expression after RT and/or immunotherapy.* Irradiation of primary tumors resulted in tumor growth hindrance and changes to the immunological microenvironment of those tumors. All irradiated tumors displayed an increase in type cDC1s. This subset of DCs is associated with the increase of CD8+ T cells in the TME. In addition, they are important for the priming and activation of these immune cells by the uptake and presentation of tumor cell antigens (Ruckett et al, 2021). This is interesting due to data that suggests DCs found in the TME are unable to effectively stimulate an immune reaction to the tumor antigens, and some have been found to suppress the T cell mediated immune response at the tumor site (Reviewed in Balkwill et al., 2012).

*RT-mediated tumor control by combinations of RT and vaccination.* Currently, cancer vaccinations have been showing promise but unfortunately, they are limited in vivo. It has been suggested that this limitation could be a result of CD8+ effector T cells, which have antitumor effects, inability to enter into the tumor tissues. Therefore, treatment of tumor cells with additional therapies, such as RT, could be very important. This study was able to demonstrate that when the tumor was irradiated after vaccination with the E7 vaccine, there was antitumor immunity against TC-1 tumors (Zhang et al., 2021).

 Effective vaccines are hard to find because the complexity of cancer makes it difficult to explore. However, the E7 vaccine used in a previous study showed that an overexpressed tumor antigen, such as E7, was demonstrated to be effective in the elimination of tumors and increased survival rates. This provides evidence that the epitopes of an overexpressed antigen specific to certain types of cancers could be used to induce T cell immunity effectively (Postow et al., 2012; Zhang et al., 2021). Thus, finding more specific tumor antigens could be important in clinical vaccine development for the treatment of cancer (Postow et al., 2012).

 In addition to the above information, combination therapy with vaccines and RT has been explored in previous studies. They were able to demonstrate the timing of the combination therapy is important in tumor elimination. Typically, the immunotherapy induces a weak antitumor effect because the immunosuppressive capabilities of the TME. The combination of RT and vaccines displayed an increase in antitumor immunity in the study. So, future research should be conducted on how immune cells respond after different treatments to gain a better understanding. This will help improve the technique and sequencing of RT and vaccine combined therapy to optimize their therapeutic effect (Zhang et al., 2021).

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