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# DESTRUCTION OF TUMOR MICROENVIRONMENT AS A PROMISING TREATMENT APPROACH IN PANCREATIC CANCER

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## რეზიუმე

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**Introduction.** Pancreatic cancer death rates continue to increase, with a very low survival for all stages combined [1]. Its incidence varies greatly across regions, which suggests that lifestyle factors play an important role in its etiology. Pancreatic cancer risk is associated with more than 50 specific risk factors with two-thirds of them potentially modifiable, such as smoking, alcohol intake, sedentary lifestyle, etc. affording a unique opportunity for preventing one of the deadliest cancers [2]. Unfortunately, pancreatic cancer was anticipated to move from the fourth to the second leading cause of cancer death in the United States by 2020. Management of pancreatic cancer depends on tumor staging. While detected early, cancer can be treated with surgery, neoadjuvant therapy, chemotherapy, radiotherapy, immunotherapy, and combination treatments [3-7]. Unfortunately, a vast amount of currently available therapeutic options almost always fail to cure PDAC patients. The insidious and quick progression of PDAC is attributed to its characteristic "Immunologically cold" environment, making pancreatic cancer very difficult to diagnose until the disease has reached an advanced stage. PDAC is infiltrated with pro-tumorigenic cells causing impaired antitumor immune response resulting in rapid disease progression and poor survival even when treated with all currently available methods. Due to no effective antitumor response tumor has an insidious course and rapidly disseminates, resulting in metastatic pancreatic ductal adenocarcinoma (mPDAC) found in most patients at the time of diagnosis. The prognosis is poorest for mPDAC regardless of innovative treatment approaches and combination therapies with a 5-year survival rate below 5%. A poor performance status, which is very common in patients with metastatic disease, leaves these patients with no effective treatment [5,8]. Currently available chemotherapies have a difficult regimen that is best reserved for fit patients who are rare exceptions at this stage of disease [9]. Dysregulation during PDAC carcinogenesis, broad heterogeneity of genetic mutations and dense stromal environment precisely fibroblasts in desmoplastic stroma compressing the blood vessels, reducing blood perfusion into the tumor makes PDAC one of the most chemoresistant cancers [10-14]. Considering the rapid upregulation of

antitumor pathways, even the therapies targeting cancer-associated molecular pathways have not given satisfactory results [8]. Immunotherapy has only limited efficacy against PDAC because of an immunosuppressive tumor-associated stroma [14,15]. Disruption of the protumorigenic immunosuppressive stroma has been described as a promising approach to treat cancer patients. Disruption of the dense and immunosuppressive stroma with ablative therapies can give us promising results in pancreatic cancer treatment. Despite the ongoing active medical effort, unfortunately, nothing effective can be suggested for mPDAC patients with poorly controlled comorbid conditions [6]. However, if left untreated, median survival in patients with metastatic disease is only 3 months [8,16]. Palliative treatment may be the only option in many cases. Furthermore, most suggested palliative treatments for metastatic disease are not effective even with the aim of prolonging survival and relieving disease-related symptoms. Thus, the development and implementation of novel PDAC treatment approaches is a crucial emergency for patients [17,18]. The scope of this review is to summarize the current concepts and newest advances in research of the tumor microenvironment destruction with RT and ATs and following immunomodulatory changes.

**Immunology of PDAC.** Approximately 50% of the cell mass of pancreatic cancer is made up of immune cells, most of which are immunosuppressive. Therefore, PDAC is also considered an "immunologically cold" tumor [19]. Immunosuppressive microenvironment includes tumor-associated macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs). These cells are responsible for the downregulation of immune response against pancreatic cancer. Macrophages are polarized to the M2 subtype that secretes IL-10 and carbon monoxide and, therefore, suppresses cytotoxic T cells [20]. MDSCs are immature monocytes and granulocytes. Cancer-derived molecular signals block the maturation of these cells into their mature counterparts. MDSCs then suppress both innate and adaptive immunity and are associated with a poorer prognosis. In most cases, pancreatic cancer is surrounded by dense connective tissue called desmoplasia [14]. Additionally, desmoplastic stroma contains pancreatic stellate cells (PSCs), which keep $CD8^{+}$-T cells away from the tumor microenvironment [21]. High infiltration of Tregs and Th17 cells causes the consequent impairment of effector, CD4+ and CD8+ cell-mediated antitumor responses [22,23]. Due to no effector response, metastases are more frequently observed in patients with decreased immune effector cells [24]. The presence of CD4+ and CD8+ T cells in the tumor has been showed to correlate with better prognosis because anti-tumor immunity cannot be activated without them [25].

Some of the immune escape mechanisms in PDAC include elevated levels of immune-suppressive tumor necrosis factor-alpha, TGF-β1, IL-10, and IL-1β [26].

Taking these premises into account, destruction of stroma to disrupt Treg-mediated immunosuppression and to block the inhibitory pathways on effector T cells seems to be potentially effective in PDAC treatment.

**Immunological aspect of RT and Ats.** Use of RT and ATs to expose these antigens and induce the immune response against the tumor has been studied over a period of time. It has been known for a long time that tumor cells release a large number of antigens, referred to as tumor-associated antigens in the form of necrotic and apoptotic tumor cells and debris [27-29].

The major goal of immune stimulation is to create immune memory and systemic response against these tumor-associated antigens, also known as an abscopal effect [30].

**RT.** The effect of radiation on immune response remains controversial with a number of studies suggesting immunosuppression, significant modulation of, or no effect at all.

Generally, immunomodulatory changes following RT include enhanced antigen presentation and tumor immunogenicity, increased production of cytokines and altered tumor microenvironment, enabling the destruction of the tumor by the immune system [31] .

Later studies suggest that lower doses of radiation have a greater potential to enhance immune responses [32]. This reflects the capability of moderate-dose radiation to enhance tumor antigen presentation, resulting in a greater diversity of antigen recognition by the antitumor T-cell response [33].

The substantial increase in the number and diversity of tumor-associated antigens can enable antigen-presenting cells and dendritic cells to stimulate a tumor-specific immune response. In addition to

tumor cells acting as the trigger, the destruction of the tumor-supporting stroma that often results from radiotherapy can also potentiate immune recognition [34].

RT is also able to induce a local antitumor immune response, potentially leading to systemic antitumor immunity having an “abscopal effect” in many tumors [35,36].

RT triggers immunogenic cell death by DNA damage and releasing damage-associated molecular patterns (DAMPs) from tumor cells. This turns the tumor cells into an “in situ vaccine” [37]. These effects promote modulation of the peptide repertoire, antigen-processing machinery components. Enhancement of MHC I expression and DC antigen presentation induces differentiation of naïve T-cells towards an effector phenotype [38,39]. Release of ’danger’ signals was also seen following radiotherapy, which stimulates the transition from nonspecific immune responses to adaptive immunity [40,41]. Radiation induces molecular alterations in the biology of the cancer cell that make the tumor more susceptible to cytotoxic-T-lymphocyte-mediated destruction [42].

These radiation-induced changes have not been well researched in pancreatic cancer.

The optimal role of radiation in immunomodulation of pancreatic cancer is unknown. Only several studies have suggested its effectiveness [43-46]. Lee et al. found that ablative RT is more effective than fractionated RT at recruiting T cells in a murine orthotopic pancreatic tumor model. Fractionated RT induced more myeloid-derived suppressor cell infiltration than ablative RT [47].

**RFA.** RFA has been successfully used to treat many tumors. As RFA is a relatively newer treatment option the definitive role of RFA for pancreatic cancer remains under investigation. Although its use in the management of unresectable pancreatic cancer is increasing [48].

RFA is a safe and effective procedure and may improve survival in patients with advanced stage pancreatic cancer. The co-author of this article (Mizandari et al.) performed percutaneous RFA in 134 patients with malignant obstructions of bile and pancreatic ducts (32 patients with pancreatic adenocarcinoma) and reported a 97% success rate of the procedure with only two patients experienced procedural technique related adverse events (contrast extravasation) following RFA [49].

RFA does not remove the tumor from the body. Instead, RFA causes tumor destruction and cytoreduction through multiple mechanisms such as coagulative necrosis, protein denaturation and generates an intense immune response. While local immune response removes the necrotic debris, and activates anticancer immunity [50-52].

Although the precise mechanism of RFA-induced immune response is not yet well established, dendritic cells (DCs) are thought to play a major role as the cells of innate immunity. RFA induces hyperthermia causing protein denaturation, vessel disruption, and cellular necrosis. Exposing antigens and activation DCs is one mechanism RFA can induce antitumor immunity [53]. Hyperthermia upregulates heat shock protein 70 (HSP-70) which then activates DCs [54]. Dendritic cells are the most potent antigen-presenting cells that recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [14].

Researchers reported the production of DAMP ingested by DC as an effect of cancer cells necrosis via RF in mice. Subsequently, matured DCs stimulate effector CD4+ T cells [55].

Brok *et. al* showed that RFA-induced DC infiltration is a more potent immune stimulator than ex-vivo generated antigen-loaded DC-based vaccines. The data revealed that upon tumor destruction by RFA, up to 7% of the total draining lymph node DC contained antigen, whereas only a few DCs from the conventional vaccine were able to reach the lymph nodes [53]. Dromi *et. al* investigated the RFA-induced immune response while treating murine urothelial tumors in mice. Their experiment revealed that RFA of the urothelial tumor produced systemic CD4+ and CD8+ T-cell responses, which were measurable in the spleens of female mice. Additionally, it was found that there are higher numbers of DCs in RFA treated tumors compared to untreated tumors. It was also shown that the nonablated tumor portion regressed in several mice due to the systemic T-cell antitumor immune response. Furthermore, primary tumor eradication was accompanied by rejection towards a second tumor implant without additional RFA. This was explained by immune memory generated by initial RFA [55].

Another research showed promising results regarding RFA-induced immune response in the management of lung cancer. Intense infiltrations of CD4+ and CD8+ T lymphocytes were found on the periphery of the RFA-treated tumor tissue, whereas the central area remained almost free of lymphocytes

in 8 days after RFA treatment. In the peripheral blood, the concentration of proinflammatory and immunostimulatory DCs was increased after RFA. Additionally, a significant rise in T-cell proliferation was detected in T-cell assays after RFA. In other words, they found that RFA-induced necrotic debris may serve as an antigen source to induce an antitumor immune response [56].

Qinglin et al. established a PDAC mouse model with tumor bilateral implants. Upregulation of PD-1 (*Pdcd1*) in CD4+ and CD8+ T cells, as well as upregulated LAG3 in T cells after RFA treatment, indicated the prevalence of T-cell exhaustion at distant tumors. It was found that RFA treatment reduced the proportions of immunosuppressive cells, including regulatory T cells, tumor-associated macrophages, and tumor-associated neutrophils, whereas increased the percentages of functional T cells in distant non-RFA tumors [51].

Faraoni et al. reported Endoscopic ultrasound guided radiofrequency ablation (EUS-RFA) immunomodulatory effect on preclinical mouse models. Reduction in tumor growth rate was seen 4 days after RFA treatment in RFA and non-RFA side contralateral tumors in mice. This reduction in size was accompanied by significant upregulation of cleaved Caspase3 expression in RFA-treated tumors and significant remodeling of the stroma. Granzyme B was significantly increased in RFA treated tumors, compared to controls. These results led to hypothesize that RFA initially promoted a strong local and systemic anti-tumor response which might have generated sustained Th1 responses and reduced secondary systemic immunosuppression [50].

Giardino et al. compared the concentration of CD4+ and CD8+ T cells before and after RFA in 10 PDAC patients. Study revealed an increase of above-mentioned T cells from the third day after treatment suggesting the activation of the adaptive response. Immunosuppressive Treg cells were not increased after the procedure despite laparotomy and heating. Myeloid DCs, that present tumor-associated antigens, increased at day 30. Circulating IL-6 was increased at day 3 after RFA but this decreased to baseline by day 30, consistent with the supposed anti-tumour effect. There was no increased concentration of essential chemokines, such as CCL-5 and SDF1, VEGF, TGF-β and TNF-α, that might be involved in tumor-growth by sustaining cancer angiogenesis and promoting tumor-associated inflammation. These immunological changes suggested general activation of adaptive response along with a decrease of immunosuppression. Furthermore, most cells showed prolonged activation some weeks after the procedure, suggesting immunomodulation rather than an inflammatory response [57].

**IRE.** IRE has been demonstrated to be a safe and effective method for locally advanced pancreatic cancer (LAPC). This procedure may be used as a tool to overcome the immunosuppressive "cold" tumor microenvironment in LAPC.

Chaobin et al. retrospectively studied 34 patients after IRE treatment. It was shown that there was a transitory decrease followed by a steady increase for CD4+ T cell, CD8+ T cell, NK cell, IL-2, C3, C4, and IgG while a reverse trend was observed for Treg cell, IL-6, and IL10 after IRE. Elevation of CD8+ T cell was associated with favorable overall survival and progression-free survival in LAPC patients after IRE. This can be explained by a stimulated host immune response which might limit the progression and invasion of the tumor, and therefore, better survival was achieved [58] .

Furthermore, Pandit et al. studied immune regulatory T cells (Tregs) which induce immunosuppression of tumors by inhibiting patients' anti-tumor adaptive immune response. They reported that IRE induced an obvious decrease in the absolute number of Treg cell in 11 patients with LAPC. IRE treatment influenced all three Tregs (CD4+CD25+, CD4+CD25+FoxP3+, and CD4+CD25+FoxP3–) compared with pancreatectomy (4 patients) [59].

**Histotripsy.** Histotripsy is a novel, non-thermal, image guided ablation modality using pulsing regimens to generate cavitation bubble clouds that lead to precise non-thermal tumor ablation that can rapidly kill cells in a targeted region with millimeter precision [60]. Several works established that there is an immune response to histotripsy ablation in many tumors, however a definite mechanism behind this response has not been established [61-63].

Hendricks et al. detected robust immunomodulatory response to histotripsy tumor ablation utilizing an *in vitro* model of pancreatic adenocarcinoma. DAMP and antigen release was demonstrated after ablation. Systemic immune response involving CD4+ and CD8+ T cell activation was confirmed with IL-2 and INFg ELISAs as well as flow cytometry. Further, changes in immune cell populations within the

tumor were consistent, there was an increase in macrophages and dendritic cells 24 hours and 7 days and in T cell populations at 7 and 14 days after treatment [64].

**Summary**

As described above, the pancreatic cancer microenvironment is insensitive to treatment, characterized by a desmoplastic stroma, acting as a physical barrier filled with immunosuppressive immune cells and molecules. Destruction of this stroma and exposition of tumor antigens might be the cause of prolonged survival of the mPDAC patients treated with ablation techniques discussed above.

Use of tumor microenvironment destruction in the management of unresectable pancreatic cancer as a potential inductor and stimulator of antitumor immunity is a relatively new field of research that may one day build a bridge between local and systemic cancer treatments and could be combined with other treatments in a multimodality approach to treating cancer.

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## DESTRUCTION OF TUMOR MICROENVIRONMENT AS A PROMISING TREATMENT APPROACH IN PANCREATIC CANCER

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### SUMMARY

Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of pancreatic cancers. Every year, we face approximately 500,000 new PDAC patients and almost the same number of deaths from this devastating disease both in men and women. The dismal prognosis can be attributed to the immunosuppressive composition of the tumor microenvironment, causing antitumor immune response inhibition, resulting in pancreatic cancer initiation, insidious and rapid progression, and dissemination. The dense desmoplastic stroma, an essential component of the cancer microenvironment, is acting as a physical barrier manifesting in treatment-insensitive pancreatic cancer. Disruption of the dense and immunosuppressive stroma with radio and ablative therapies gives us promising results as the possible inductor and enhancer of an antitumor immune response. In this review, we discuss stromal-targeting ablation methods along with radiotherapy as a dense stromal environment destruction tool and activator of antitumor immune response in pancreatic cancer patients.

**Keywords:** Pancreatic ductal adenocarcinoma (PDAC), immunomodulation, ablative therapy (AT), radiofrequency ablation (RFA), irreversible electroporation (IRE), histotripsy, radiotherapy (RT)