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The effects of irreversible electroporation triggering anti-tumor immunity and the value of its combination with immunotherapy

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A B S T R A C T

Recently, interventional ablation techniques have gained prominence in tumor treatment guidelines and complement traditional approaches, such as surgery, chemotherapy, and radiotherapy. Conventional ablation techniques, such as microwave, radiofrequency, and cryoablation, have been used; however, they have certain limitations, including the risk of damaging surrounding normal tissues and the heat sink effect caused by tumor blood flow.[1] Irreversible electroporation (IRE), an ablation technology independent of thermal energy, is a promising alternative.[2] Clinical studies have demonstrated IRE's efficacy in treating tumors, such as pancreatic and liver tumors.[3] Recent research has shown that IRE can elicit specific anti-tumor immune responses in the body.[5] IRE also plays a crucial role in eliminating residual tumor cells postoperatively and preventing tumor recurrence.

1. Comparison of IRE and traditional ablation

Traditional tumor ablation techniques such as microwave ablation, radiofrequency ablation, and cryoablation are commonly used in clinical practice. In cases of primary liver cancer, the guidelines recommend a combination of transcatheter arterial chemoembolization (TACE) with thermal ablation. Cryoablation, in particular, allows for real-time monitoring and precise control of the ablation area by visualizing ice ball formation using computerized tomography, magnetic resonance imaging, and ultrasound scans.

However, traditional ablation methods exhibit limited selectivity. Owing to its relatively low selectivity, the ablation process inevitably affects the surrounding normal tissues, including blood vessels and bile ducts. Furthermore, the heat sink effect can diminish the ablation efficacy when blood vessels are present near the target lesion because the flowing blood carries away a portion of the heat.[1]

In contrast, irreversible electroporation (IRE) selectively affects the phospholipid bilayer of the cell membrane while having minimal impact on other molecules, such as membrane proteins.[2] IRE disrupts the cell membrane, allowing the cell contents to flow out and extracellular substances to enter the cell by creating irreversible nanoscale pores on the target cell membrane. Compared to traditional methods, IRE significantly preserves the integrity of blood vessels, bile ducts, and connective tissues. Therefore, IRE is particularly suitable for tumors near high-risk areas in the body, such as adjacent blood vessels, bile ducts, and porta hepatis.[3] Over the past decade, IRE has emerged as an effective adjunct treatment for pancreatic cancer, particularly for patients with locally advanced pancreatic cancer (LAPC).[4] However, the potential risk of complications associated with IRE should not be overlooked.[5]

Both traditional ablation techniques and IRE induce apoptosis and necrosis of tumor cells, leading to the release of tumor antigens that initiates an immune response; however, the extent of immune response activation varies.[6] Shao et al. compared the immune response activation effects of thermal ablation, cryoablation, and IRE.[7] They found that IRE released the highest amounts of proteins and antigens, particularly TRP-2, followed by cryoablation and thermal ablation, respectively. Cryoablation resulted in the release of many undenatured tumor proteins, because protein denaturation is irreversible at high temperatures but reversible at low temperatures. Therefore, the tumor proteins released by cold treatment were considered the best “quality.” However, the subsequent immune response was more significantly activated by IRE than cryoablation or thermal ablation, indicating that IRE released the highest “quantity” of tumor proteins. The “quantity” and “quality” of tumor proteins released after ablation significantly impacted the initiation of the immune response. Additionally, IRE causes minimal damage to the peripheral blood vessels to allow rapid release of tumor antigens into the bloodstream and facilitate the entry of immune cells into the ablation area. These factors contribute to the superior ability of IRE to activate subsequent immune responses in contrast to traditional ablation techniques, as shown in Table 1.

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Table 1
Comparison of IRE and traditional ablation.

Ablation Modality	Immune Activation	Quality of Antigens	Quantity of Antigens
Thermal Ablation	Moderate	Moderate to Low	Moderate
Cryoablation	Moderate to High	High	Moderate to High
Irreversible Electroporation (IRE)	High	High	High

2. IRE-induced anti-tumor immune function

2.1. IRE-induced cell apoptosis

In contrast to surgery, IRE causes tumor cell death by releasing DAMPs (damage-associated molecular patterns) into the bloodstream.[8–11] DAMPs, such as HMGB1 and ATP, stimulate dendritic cells, triggering immune responses by presenting tumor-specific antigens. HMGB1 promotes M1 polarization of macrophages, which is crucial in postoperative immunotherapy for liver and pancreatic cancers.[12] IRE also improves the infiltration of cytotoxic T lymphocytes (CTL) into tumors, reduces regulatory T cells (Tregs), and inhibits myeloid-derived suppressor cells (MDSC), contributing to local tumor destruction and systemic antitumor activation similar to in situ tumor vaccination. IRE-induced apoptosis increases caspase-3 activity, which reflects tumor cell death. This immunogenic cell death releases or exposes DAMPs, and activates maturation of immature dendritic cells and prime naïve T cells into tumor-killing CTL. These CTL eradicate both local and distant tumor cells. Moreover, differentiated T cells can become memory T cells, offering long-term protection against tumor recurrence.

The ability of IRE to induce tumor cell apoptosis and release DAMPs demonstrates its potential as a therapeutic strategy for triggering strong antitumor immune responses and preventing tumor recurrence. Additionally, IRE has the potential to strengthen the body's defense against cancer by promoting immune cell activation.

2.2. Released antigenic proteins

In a study by Veronica M, high-frequency IRE was applied to a 4T1 breast cancer mouse model and newly generated 4T1 tumor antigens were detected in mouse serum.[13] These tumor antigens can be presented to T cells through dendritic cells, leading to the generation of cytotoxic T lymphocytes (CTL) and memory T cells. This mechanism plays a crucial role in the immune response against tumor cells.

Tumor antigens are often concealed within tumor cells, preventing their recognition by immune cells and facilitating immune evasion. However, IRE can expose hidden antigens and trigger the activation of a specific immune system. This activation leads to an antitumor immune response that effectively slows tumor progression, mimics the effects of in situ tumor vaccination, and reduces the risk of tumor recurrence.

2.3. Immunoregulation

IRE can increase the production of Th1 cytokines, including IFN-y, TNF-a, IL-1a, IL-2, IL-12P70, and IL-3. These cytokines have been shown to inhibit cancer growth, and their elevated levels indicate an enhanced tumor immune response. Following IRE treatment, levels of the inhibitory cytokine IL-10 decreased. In a liver cancer model, there was a temporary decrease in peripheral blood lymphocytes, particularly CD4+T cells, one day after IRE. However, three to seven days after the procedure, there was an increase in activated CD8+T cells in the peripheral blood, suggesting an enhanced immune response. Tregs(regulatory T cells) in the peripheral blood decreased 3–14 days after IRE, indicating a reduction in immunosuppression. However, one month after the procedure, Tregs showed a significant increase, leading to a decline in the intensity of the immune response. These molecular-level changes demonstrate that IRE initially strengthens the postoperative tumor immune response, followed by gradual weakening, thus providing a window of opportunity for tumor cell immunotherapy.[14]

2.4. CD8+T cells

He et al.[15] discovered that after IRE treatment, there was increased infiltration of CD8+T cells into the tumor region, leading to a localized immune response. To enhance the specific immune response, Dai et al.[16] introduced IRE-treated tumor cell lysates as a vaccine into mice with existing tumors after IRE treatment. Subsequently, the tumor cells were re-inoculated. New tumors were not observed after some time; however, significant infiltration of CD8+T cells into the original tumor-infiltrated area was observed. This suggests that IRE effectively stimulates CD8+T cell-mediated immunity.

Researchers have discovered a crucial role of CD8+T cells in the host immune response against malignant tumors. After dendritic cells present tumor antigens, they stimulate CD8+T cells to proliferate and differentiate into memory T cells and cytotoxic T lymphocytes (CTL). These differentiated T cells circulate throughout the body via the bloodstream, specifically targeting and eliminating corresponding tumor cells. Notably, tissue-resident $CD8^+$ memory T-cells (TRMS) play a significant role.[17–20] TRMS permanently resides in the primary tumor site and surrounding tissues and promptly initiates an immune response when secondary tumors are likely to develop in these areas. Brandon et al.[21] treated a mouse model of prostate cancer with an anti-CTLA-4 immune checkpoint inhibitor (ICI) following IRE. They observed a substantial expansion of tumor-specific CD8+T cells in the blood, tumor area, and non-lymphoid tissues of the treated mice. Parabiosis studies confirmed the effective formation of TRMS after IRE and ICI treatment, with TRMS residing in the tissues surrounding the tumor lesions. This mechanism can impede tumor progression and prevent subsequent adverse events.

2.5. Regulatory T cells (Tregs)

Tregs, a subset of CD4+T cells with a significant immunosuppressive effect, prevent the immune system from attacking normal tissues. In recent years, numerous studies have demonstrated elevated levels of Tregs in the peripheral blood and tumor microenvironment of patients with pancreatic, lung, colorectal, and other cancers. Treg cell accumulation protects tumors from immune system attack. Scheffer et al.[22] discovered that IRE treatment resulted in a temporary decrease in Tregs and a transient increase in PD-1+T cells. These changes enhanced the immune response of tumor-specific T cells by targeting the pancreatic cancer-associated antigen WT1 in two-thirds of the patients. Guo et al.[23] observed a temporary reduction in Tregs after IRE treatment. These findings suggest that IRE can attenuate or eliminate the immunosuppressive effects of Tregs, thereby facilitating an enhanced immune response.

2.6. Innate immunity

Lopez-Ichikawa et al.[24] discovered that tissue repair processes were expedited following IRE treatment, potentially owing to the delayed but robust infiltration of neutrophils. These neutrophils facilitated the generation of pro-reparative Ly6Clo monocytes/macrophages at the site of injury after IRE treatment, thereby promoting the healing of the damaged area.

3. Combination of IRE and immunotherapy

3.1. Anti-PD1

PD-1 receptor is located on the surface of cytotoxic T lymphocytes (CTLs), while the PD-L1 receptor is present on the surface of tumor cells.

The interaction between PD-1 and PD-L1 inhibits the antitumor activity of CTLs when CTLs recognize major histocompatibility complexes (MHC) on the tumor surface via their T-cell receptors (TCRs). This is one of the mechanisms by which tumors evade the immune system.

Programmed death-1 (PD-1) inhibitors act on the PD-1 receptor, preventing its binding to PD-L1. This inhibition disrupts the immune response of tumors and promotes tumor cell apoptosis.

Zhao J et al.[25] treated mouse pancreatic ductal adenocarcinoma (PDAC) in situ using IRE, anti-PD-1 therapy, and their combination. The median survival times after treatment were 6, 8, 11.5, and 31.5 days in the control, anti-PD-1 therapy, IRE treatment, and IRE + anti-PD-1 treatment groups, respectively. The median survival time in the IRE + anti-PD-1 group was significantly longer than that in the other groups. At the 60-day endpoint, four (36%) mice in the IRE + anti-PD-1 group were still alive and showed no palpable tumors. Researchers believe that combining IRE and anti-PD-1 therapy plays a crucial role in inducing a T-cell immune memory response. Furthermore, IRE can modulate the stromal environment of pancreatic cancer cells by increasing microvascular density and vascular permeability, softening the dense extracellular matrix, and alleviating hypoxia. These modifications in the tumor microenvironment improve the immunosuppressive state of the tumor and create an opportunity for combined immunotherapy.

Oncologists have successfully used anti-PD-1 therapy in combination with IRE to treat patients with locally advanced pancreatic cancer and have achieved excellent therapeutic outcomes. He et al.[26] reported that the combined use of IRE and toripalimab (a PD-1 monoclonal antibody) resulted in overall survival (OS) of 44 months and progression-free survival (PFS) of 27 months, which were significantly longer than the OS (23 months) and PFS (10 months) of patients treated with IRE alone. These findings demonstrate the effectiveness of combining IRE with immunotherapy and provide a viable treatment strategy for patients with tumors.

3.2. Anti-Ox40

Ox40 is primarily expressed on the surface of activated T cells. It enhances the tumor-killing effect of T cells when paired with Ox40L on antigen-presenting cells. The expression level of Ox40 is positively correlated with T-cell levels and serves as an independent prognostic factor for pancreatic cancer. The group with high Ox40 expression exhibited significantly longer overall postoperative survival than those with low Ox40 expression.

Anti-Ox40 is a drug functionally similar to Ox40L, which pairs with Ox40 and activates downstream molecules to enhance the tumor-killing effect of T cells. In our previous study,[27] we compared the therapeutic effects of Anti-Ox40 and IRE, and their combination in mice with pancreatic cancer. The control, Anti-Ox40, and IRE groups had a total survival time of approximately 22, 24, and 51 days, respectively. However, tumor recurrence occurred approximately 17 days after surgery. The IRE combined with the Anti-Ox40 group exhibited a survival time of over 120 days, with 80% of the tumors being eradicated; therefore, we believe that the postoperative immune effects of IRE increase the number of CD8+T cells and the total amount of Ox40 receptors. This, in turn, increases the successful pairing of Anti-Ox40 drugs with Ox40 receptors, thereby strengthening the postoperative antitumor immune response induced by IRE, and inhibiting tumor recurrence and metastasis. These findings provide evidence and novel insights into combining IRE and immunotherapy to treat pancreatic cancer and other tumors.

3.3. Anti-CTLA-4

In a study by Brandon et al.,[21] combining IRE with an anti-CTLA-4 immune checkpoint inhibitor (ICI) increased tumor-specific $CD8^+$ T cells in the blood, tumor, and non-lymphoid tissues. The treatment also induced tissue-resident $CD8^+$ memory T cells (TRMS) in the tissues surrounding the tumor lesions, thereby preventing tumor progression and adverse events.

Table 2
The effects of combination treatment.

Combination Treatment	Effects
IRE + Anti-PD-1	- Enhanced tumor-specific $CD8^+$ T cell response - Increased recognition and presentation of tumor antigens by antigen-presenting cells - Improved postoperative anti-tumor immune response - Inhibition of tumor recurrence and metastasis
IRE + Anti-OX40	- Enhanced killing effect of T cells on tumors - Increased Ox40 receptor expression - Strengthened postoperative anti-tumor immune response - Inhibition of tumor recurrence and metastasis
IRE + Anti-CTLA-4	- Increased tumor-specific $CD8^+$ T cell response - Presence of tumor-related memory-like T cells in tumor microenvironment - Prevention of tumor progression and adverse events - Enhanced tumor immune response

The effects of the combination treatments above are shown in Table 2.

3.4. Other drugs

TLR agonists such as TLR7 and TLR3/9 have been investigated with IRE to enhance the immune response against tumors. Narayanan et al.[28] demonstrated that the intratumoral injection of TLR7 agonists improved the tumor immune response when combined with anti-PD-1 in head and neck tumor mouse models. Similarly, Babikr et al.[29] showed that combining IRE with TLR3/9 agonists and anti-PD-1 therapy regulates the immune cell profile in a lymphoma mouse model, leading to immunotolerance conversion. These findings suggest that TLR agonists can reduce tumor microenvironment inhibition.

Tumor-Associated Neutrophils (TANs) play a complex role in the antitumor process. Peng et al.[30] successfully transformed TANs from an immunosuppressive N2 phenotype to an antitumor N1 phenotype using TGF-β inhibitors loaded into nanoparticles. This transformation enhanced the efficacy of IRE and anti-PD-1 combination therapy for pancreatic cancer, and induced a long-term anti-tumor memory response.

Stimulator of Interferon Genes (STING) is a crucial tumor immunomodulatory factor in the innate immune recognition of tumors and $CD8^+$ T cell activation. Go et al.[31] observed that the combination of IRE and STING agonists significantly inhibited tumor growth in LLC tumor-bearing mice without any noticeable adverse reactions.

Oncolytic viral therapy, which utilizes viruses to target and destroy tumor cells, has shown promise in combination with IRE. Sun et al.[32] demonstrated that IRE facilitates the infection of pancreatic cancer cells by the M1 oncolytic virus. This combination therapy inhibited tumor proliferation and significantly prolonged the survival of the mice, providing a potential strategy for treating pancreatic cancer.

Overall, these approaches aim to enhance the immune response, modulate the tumor microenvironment, and improve treatment outcomes in combination with IRE.

Declaration of competing interest

Xiaoyi Ding is an editorial board member for Journal of Interventional Medicine and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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